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STUDIES ON RESISTANCE OF APPLE AND OTHER ROSACEOUS PLANTS TO FIRE BLIGHT [1]

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INTRODUCTION

Studies of fire-blight resistance [3] were conducted along three lines: (1) The occurrence of fire-blight resistance in certain rosaceous plants; (2) the effects of certain partially controlled environmental conditions on fire-blight resistance in apple (*Malus* spp.) shoots; and (3) the relation of certain internal host conditions to resistance to fire blight.

The literature reveals that differences in the invasion of certain host plants by the fire-blight pathogene, *Erwinia amylovora* (Burr.) Com. S.A.B.[4] were observed by some of the earliest investigators of the disease. Burrill (*5*) stated that, as a group, apple varieties were more resistant to fire blight that were pear varieties. Arthur (*1*) observed wide varietal differences in fire-blight resistance among both apple and pear. These earlier observations have been followed by numerous reports on varietal resistance of both apple and pear to fire blight, including those by Waite (*29*), Crandall (*7*), Whetzel (*30*), Jones (*12*), and Stewart (*25*). These reports, with further field observations, were the basis for classifications of certain commercial apple varieties according to their relative fire-blight resistance by several writers, including Orton and Wood,[6] Brooks (*4*), and Miller (*16*). With the reports on varietal resistance, considerable reference is made to the effects of certain weather conditions and cultural practices on the fire-blight resistance of plants under orchard conditions (*4*, *12*, *16*, *25*, *30*).

The need for further experimental work on fire-blight resistance has been emphasized in several investigations, including those by Reimer (*22*), Brooks (*4*), and Miller (*16*). These writers showed the importance of employing controlled conditions in studies on fire-blight resistance and the necessity of using a standardized technic in testing the relative resistance of plants to the disease. These considerations have been given attention in the studies here reported.

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[3] Since the experiments reported in this paper do not furnish a basis for satisfactory segregation of the effects of susceptibility from those of predisposition, it is not possible to distinguish between them in the discussion. Therefore the term "resistant" is used in a relative sense throughout this paper to refer to plants which blighted slightly, and "susceptible" to refer to those which blighted severely.

[4] For the synonymy of *Erwinia amylovora* see Elliott (*8*, p. *19*).[5]

[5] Reference is made by number (italic) to Literature Cited, p. 312.

[6] ORTON, C. R., and WOOD, J. I. DISEASES OF FRUIT AND NUT CROPS IN THE UNITED STATES IN 1923. U.S Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. Sup. 33: 70-80. 1924.

MATERIALS AND METHODS

Plants for these studies were of two kinds: (*1*) Potted plants which were grown in a greenhouse and used exclusively in some phases of the work, and (2) orchard trees, which supplied an abundance of resistant and susceptible terminal shoots.

For the greenhouse work nursery trees of suitable size were pruned and placed in soil in 8-inch pots or galvanized-iron cans. The soil used was composed of 4 parts of well-rotted sod and 1 part of sand. After the plants were potted they were kept in a cool basement for approximately 2 weeks and were then placed under the desired conditions in a greenhouse. Unless otherwise stated, the plants were grown in the same greenhouse with average illumination and with a temperature held at approximately 24° C. The period during which plants were grown in the greenhouse extended from January 15 to July 15 of each year.

Uniformity in age of shoots was found to be of prime importance in duplicating results. To obtain shoots of approximately the same age to be used in a given series of experiments, only the shoots which appeared on the plants during a period of 5 days were allowed to continue growth. This procedure necessitated the removal of the earlier shoots until essentially all of the plants had begun growth. When this condition was reached no more shoots were removed during the following 5 days. At the end of this period, from 1 to 8 shoots on each plant were selected for continued growth. The remaining shoots on each plant, and those which appeared on later dates, were removed. The age of the shoots was reckoned from the day on which the shoots were selected for continued growth.

Uniformity in the growth condition of shoots on the plants was found to be of great importance in obtaining uniformity in results. The rate and condition of growth of shoots were found to vary considerably with variations in the number of shoots on each plant. The number of shoots which a given plant would support varied with the species or variety concerned. In *Amelanchier*, *Cotoneaster*, and *Crataegus* species from 3 to 8 shoots were permitted on each plant; in *Malus* and *Sorbus* species from 1 to 5. These plants, with varying numbers of shoots, were used in the same or parallel experiments. In the commercial apple varieties from 1 to 3 shoots were allowed to develop on each tree, 2 being the usual number. Three shoots were permitted only when they were to be used at a relatively early stage, since it was found that, under the conditions employed, 3 shoots on the same plant would not maintain a uniform condition of growth over a long period. However, most of the trees would support 2 shoots in a satisfactorily uniform condition of growth as long as desired. With but few exceptions, only apple trees bearing the same number of shoots were employed in the same or parallel experiments.

The field material consisted of 18-year-old trees of a limited number of apple varieties growing on the university farm, Madison, Wis. Only the terminal growth on trees in a practically uniform condition of vegetative growth was used.

The method employed for testing the relative fire-blight resistance of plants was as follows: Plants with shoots of the desired age were selected and tagged. The total length of each shoot, in inches, was then recorded. Following this, each shoot was inoculated with

Erwinia amylovora at one point about 1 cm from the tip. Two methods of inoculation were employed. (1) A water suspension of *E. amylovora* was prepared by washing the bacterial growth from the surfaces of nutrient-dextrose-agar slants which had been incubated for 24 to 48 hours at 24° C. The suspension was prepared in the proportion of 1 test-tube slant of the pathogene to 5 cc of sterile distilled water. A portion (less than 0.1 cc) of the bacterial suspension was injected into each shoot by means of a 2-cc hypodermic syringe. (2) A portion of bacterial growth from an agar slant was placed on the surface of each shoot at the selected point. The shoot was then pricked, through the bacterial mass, with a dissecting needle. The essential features of this method of inoculation have been employed by several authors (*4*, *16*, *17*).

The first method of inoculation was used in the earlier work, but the second method proved to be the more practical and was used in the major portion of the investigation. The method of inoculation was not varied in the same experiment. The two methods of inoculation appeared to be equally effective in inducing fire-blight infections.

The progress of fire blight in each inoculated shoot was noted by measuring the length of the fire-blight lesion, in inches, at definite intervals up to 43 days after inoculation. Measurements after 43 days did not appear to be necessary or practical as lesions rarely should advance thereafter. Soon after macroscopic blight appeared on a given shoot the blighted portion frequently dried rapidly and shrank; this made it difficult to get an accurate, direct measurement of the portion of the original shoot length which was blighted. This source of error was avoided by measuring the length of the healthy portion of the shoot, which was then subtracted from the total length of the shoot taken at the time of inoculation.

The time after inoculation at which advance of the fire-blight lesions ceased varied considerably among varieties and among different inoculation series.

The cultures of *Erwinia amylovora* which were used in these studies were obtained as follows:[7] Culture A was the progeny of a single cell picked from a culture which was originally isolated from a blighted Wealthy apple limb. This limb was collected at Madison, Wis., in July 1929. Culture B was isolated from a blighted pear limb (variety unknown) collected at Fayetteville, Ark., in July 1930. Culture C was isolated from a blighted apple limb (variety unknown) collected at Logan, Utah, in October 1930. Neither culture B nor C was the progeny of a single cell. Unless otherwise stated, culture B was used in the studies here reported. This culture was used because early unreported tests indicated that it was one of the most virulent in a collection of approximately 25 obtained from different sections of the United States.

A moist chamber, described by Keitt and Jones (*14*), in which an atmospheric humidity approximating saturation could be maintained at a controlled temperature, was employed in these studies. In part of the work the chamber was equipped with three 300-watt mazda lamps to provide light for the plants while in the chamber.

When controlled soil moistures were desired, the moisture-holding capacity of the soil was determined by the Wolff method (*32*, *v. 1*, *p. 150*). Soil tubes 2 inches in diameter and 8 inches in height were used.

[7] The writer wishes to express his thanks to Drs. H. L. Blood and H. R. Rosen for collecting the plant material from which cultures B and C were isolated.

THE OCCURRENCE OF FIRE-BLIGHT RESISTANCE IN CERTAIN ROSACEOUS PLANTS

The pathogenicity of *Erwinia amylovora* on a large number of plants has been established (*18*, *24*, *26*). Reimer (*22*) made an extensive study of the relative fire-blight resistance of a large number of pear species and varieties. However, comparatively little work has been done on the relative resistance of other hosts to the pathogene. Data on fire-blight resistance in certain apple varieties were reported by Stewart (*25*), Brooks (*4*), and Miller (*16*).

In the investigations here reported, a study was made of the relative fire-blight resistance of available plants growing under orchard conditions, and such plants as could feasibly be obtained and grown in the greenhouse under partially controlled conditions.

STUDIES ON VARIOUS ROSACEOUS SPECIES

The fire-blight resistance of approximately 85-day-old shoots of 31 rosaceous species was studied in a single inoculation series. The plants of each of 21 of the species were divided into two groups with approximately the same number of shoots in each group. The average number of shoots in each group was 5.5. The actual numbers employed are given in figure 1. One group of shoots of each of the 21 species was inoculated with *Erwinia amylovora*, culture B, and the other with culture C. The trees were kept in a moist chamber (*14*), at 24° C., for 18 hours immediately preceding and 24 hours immediately following inoculation. In the manner previously described, measurements were made of the fire-blight lesions which developed on the shoots.

Wide differences in fire-blight resistance were found among the various rosaceous species studied (fig. 1). In the shoots inoculated with culture B these differences ranged from no infections on *Crataegus prunifolia* to a very high degree of susceptibility in *C. oxyacantha*, on which an average of 80 percent of the average shoot length blighted. Differences in resistance to blight were more marked among species of *Crataegus* than they were among species of different genera. On an average, the representatives of the genus *Malus* were the least resistant of all to the disease.

The relative fire-blight resistance of the different species took the same general order from inoculations with the different cultures of *Erwinia amylovora*. However, all of the species except two, *Crataegus tomentosa* and *Malus malus*, showed a higher degree of resistance to invasion by culture C than by culture B. In two species, namely *C. arnoldiana* and *C. grayana*, the shoots employed were completely resistant to infection by culture C, although comparable shoots were substantially infected by culture B. It is not concluded from these data that *C. arnoldiana* and *C. grayana* were immune to culture C, for it is highly probable that infections with this culture would have been obtained if younger shoots had been employed. The results do, however, bear out the difference in pathogenicity between the two cultures. Similar differences in pathogenicity were noted by Stewart (*25*) and Pierstorff (*18*).

STUDIES ON VARIOUS APPLE VARIETIES

Following the work on various rosaceous species it seemed desirable to measure the fire-blight resistance of a number of commercial apple varieties growing under orchard and greenhouse conditions.

Plants Growing Under Orchard Conditions

Studies of the fire-blight resistance of the new terminal growth on 18-year-old Northwestern Greening, McIntosh, Wealthy, and Yellow Transparent apple varieties were made on trees growing under or-

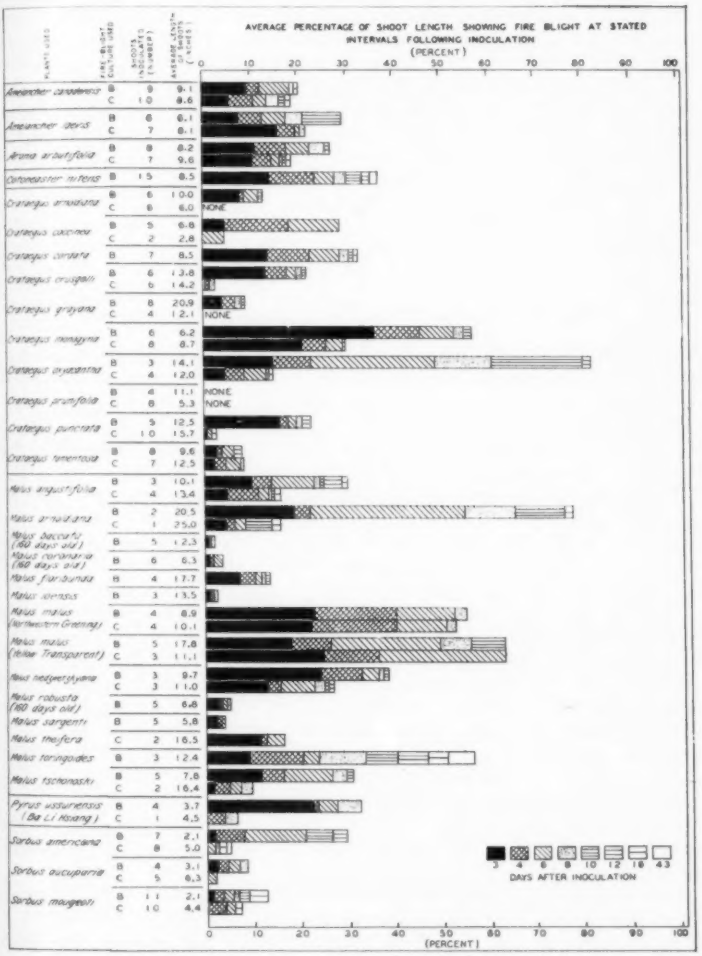
AVERAGE PERCENTAGE OF SHOOT LENGTH SHOWING FIRE BLIGHT AT STATED INTERVALS FOLLOWING INOCULATION
(PERCENT)
0 10 20 30 40 50 60 70 80 90 100
PLANT USED
FIRE-BLIGHT CULTURE USED
SHOOTS INOCULATED (NUMBER)
AVERAGE LENGTH OF SHOOTS (INCHES)
Amelanchier canadensis B 9 9.1
C 10 8.6
Amelanchier laevis B 8 6.1
C 7 8.1
Aronia arbutifolia B 8 8.2
C 7 9.6
Cotoneaster tomentosa B 15 8.5
Crataegus arnoldiana B 6 10.0
C 8 6.0 NONE
Crataegus coccinea B 5 6.8
C 2 2.8
Crataegus cordata B 7 8.5
Crataegus crusgalli B 6 13.8
C 6 14.2
Crataegus grayana B 8 20.9
C 4 12.1 NONE
Crataegus monogyna B 6 6.2
C 8 8.7
Crataegus oxyacantha B 3 14.1
C 4 12.0
Crataegus prunifolia B 4 11.1 NONE
C 8 5.3 NONE
Crataegus punctata B 5 12.5
C 10 15.7
Crataegus tomentosa B 8 9.6
C 7 12.5
Malus angustifolia B 3 10.1
C 4 13.4
Malus arnoldiana B 2 20.5
C 1 25.0
Malus baccata (180 days old) B 5 12.3
Malus coronaria (180 days old) B 6 8.3
Malus floribunda B 4 17.7
Malus sieversii B 3 13.5
Malus malus (Northwestern Greening) B 4 8.9
C 4 10.1
Malus malus (Yellow Transparent) B 5 17.8
C 3 11.1
Malus niedzwetzkyana B 3 9.7
C 3 11.0
Malus robusta (180 days old) B 5 6.8
Malus sargenti B 5 5.8
Malus theifera C 2 16.5
Malus toringoides B 3 12.4
Malus tschonoskii B 5 7.8
C 2 16.4
Pyrus ussuriensis (Ba Li Hsiang) B 4 3.7
C 1 4.5
Sorbus americana B 7 2.1
C 8 5.0
Sorbus aucuparia B 4 3.1
C 5 8.3
Sorbus meugeoti B 11 2.1
C 10 4.4
3 4 6 8 10 12 18 43
DAYS AFTER INOCULATION
0 10 20 30 40 50 60 70 80 90 100
(PERCENT)

FIGURE 1.—Relative fire-blight resistance of approximately 85-day-old shoots of various rosaceous plants to culture B, from Arkansas, and culture C, from Utah.

chard conditions. The trees had vigorous vegetative growth and were in fruit. Shoots approximating the average length and condition of growth were selected for study. Twenty-five healthy shoots of each variety, about equally divided between two trees, were artificially inoculated with *Erwinia amylovora* on each of the following dates

during the summer of 1931—June 20, July 1, July 16, and August 1. Approximately 30 days after the inoculations, records were made of the extent of fire-blight development in the shoots.

Results of these studies (fig. 8, *F*), which are referred to again on page 297, show that considerable differences in fire-blight resistance occurred among the four varieties studied. Differences resulting from the inoculations made June 20 ranged, on an average, from a negligible amount of blight on Northwestern Greening to relatively high susceptibility in Yellow Transparent, on which lesions involved an average of 20 percent of the average shoot length. McIntosh and Wealthy were in an intermediate class, with McIntosh the more resistant. Following June 20, an increase in fire-blight resistance took place in all of the varieties. From inoculations made on July 1 no infections were obtained on Northwestern Greening and McIntosh, and only a slight amount of blight was obtained on Wealthy, while an average of 4 percent of the shoot lengths blighted on Yellow Transparent. No infections were obtained on any of the varieties from inoculations made on July 16 and August 1.

Plants Growing Under Greenhouse Conditions

Studies were made in the greenhouse of the relative fire-blight resistance of 25 apple varieties. In 18 of the varieties 85-day-old and 137-day-old shoots were employed; in 6 only 85-day-old shoots were available; and in 1, only 137-day-old shoots were used. The 85-day-old shoots of all of the varieties had ceased rapid elongation, but the apical portions of most of the shoots were still succulent. The 137-day-old shoots had in all varieties ceased elongation, and the terminal buds had formed. Tests of the fire-blight resistance of shoots of both ages were made at the same time under identical conditions. The number of shoots of each variety employed in each age series ranged from 4 to 24. The average number of shoots employed in each age series was 8.5. The trees were given no moist treatment either before or after inoculation.

Results of the studies described above (fig. 2) show that wide differences in fire-blight resistance occurred among the apple varieties employed. In the groups of plants with 85-day-old shoots, Northwestern Greening, Arkansas, and Red Astrachan were very resistant, as indicated by low percentages of shoot lengths blighted at the end of 43 days (8, 6, and 6 percent, respectively). Yellow Transparent and Autumn Strawberry were very susceptible, as indicated by high percentages of shoot lengths blighted (50 and 49 percent, respectively). Substantial differences in resistance occurred among many of the other varieties studied.

An increase in fire-blight resistance took place with an increase in age of the shoots in those varieties in which shoots of different ages were employed. For example, in the 85-day-old shoots of Yellow Transparent, the blight lesions involved an average of 50 percent of the average shoot length, while in the 137-day-old shoots they involved only 11 percent, indicating a marked increase in resistance with an increase in the age of the shoots. Similar increases in resistance, although less pronounced in many cases, occurred in all of the other varieties studied. In Ben Davis the increase was expressed only in a

retardation of the rate of advance of the lesions on the older as compared with that of those on the younger shoots.

It was not found possible to make further inoculation tests on all of these apple varieties. However, numerous tests which are described in the following pages were made on Northwestern Greening, Wealthy, and Yellow Transparent. Results of these tests support those in Figure 2 for the three varieties named. The results are also in agreement with the studies on orchard trees.

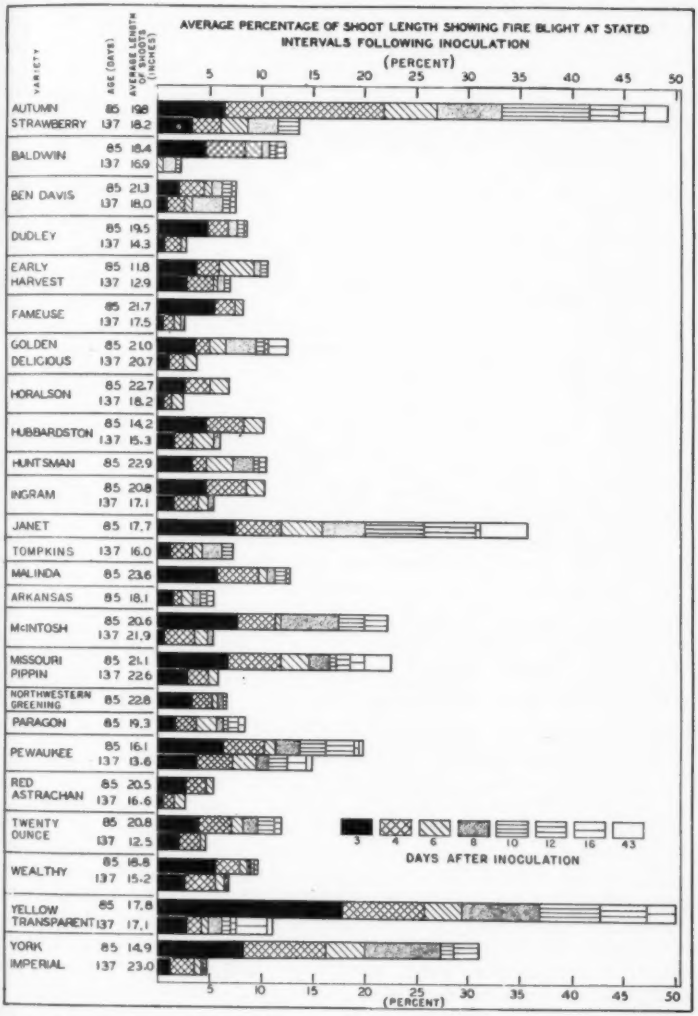
AVERAGE PERCENTAGE OF SHOOT LENGTH SHOWING FIRE BLIGHT AT STATED INTERVALS FOLLOWING INOCULATION
(PERCENT)
VARIETY
AGE (DAYS)
AVERAGE LENGTH OF SHOOTS (INCHES)
AUTUMN STRAWBERRY 85 19.8 137 18.2
BALDWIN 85 18.4 137 16.9
BEN DAVIS 85 21.3 137 18.0
DUDLEY 85 19.5 137 14.3
EARLY HARVEST 85 11.8 137 12.9
FAMEUSE 85 21.7 137 17.5
GOLDEN DELICIOUS 85 21.0 137 20.7
HORALSON 85 22.7 137 18.2
HUBBARDSTON 85 14.2 137 15.3
HUNTSMAN 85 22.9
INGRAM 85 20.8 137 17.1
JANET 85 17.7
TOMPKINS 137 16.0
MALINDA 85 23.6
ARKANSAS 85 18.1
McINTOSH 85 20.6 137 21.9
MISSOURI PIPPIN 85 21.1 137 22.6
NORTHWESTERN GREENING 85 22.8
PARAGON 85 19.3
PEWAUKEE 85 16.1 137 13.6
RED ASTRACHAN 85 20.5 137 16.6
TWENTY OUNCE 85 20.8 137 12.5
WEALTHY 85 16.8 137 15.2
YELLOW TRANSPARENT 85 17.8 137 17.1
YORK IMPERIAL 85 14.9 137 23.0
3 4 6 8 10 12 16 43
DAYS AFTER INOCULATION
(PERCENT)

FIGURE 2.—Relative fire-blight resistance of various apple varieties growing in the greenhouse.

EFFECT OF CERTAIN PARTIALLY CONTROLLED ENVIRONMENTAL CONDITIONS ON FIRE-BLIGHT RESISTANCE IN APPLE SHOOTS

Following the tests on varietal resistance of apple to fire blight, studies, which extended over a period of 3 years, were made of the effect of various partially controlled environmental conditions on resistance to fire blight in apple shoots. Correlations of certain weather conditions and cultural practices with the natural occurrence of fire blight in orchard trees have been observed by many investigators, including Arthur (*1*, *2*), Waite (*29*), Whetzel (*30*), Whetzel and Stewart (*31*), Jones (*12*), Stewart (*25*), and Brooks (*4*). Considerable difficulty is encountered, however, in attempting to appraise individual environmental factors in their relation to fire-blight resistance under natural conditions. The simultaneous variation of many factors, such as temperature and humidity, which normally occur during periods of precipitation, is a constant obstacle to such correlations. Furthermore, the wide variations in local distribution of fire-blight inoculum lead to considerable variations in the natural occurrence of fire blight (*9*, *16*, *28*), thus making it difficult to distinguish between resistance and susceptibility to disease by introducing the factor of avoidance of disease.

The importance of employing controlled conditions in evaluating various factors in their relation to fire-blight resistance has been emphasized by the work of Stewart (*25*), Brooks (*4*), Miller (*16*), and Tullis (*28*). Therefore, a study was made of the effects of partially controlled soil nutrients, temperature, and moisture on fire-blight resistance in a limited number of apple varieties. These conditions were studied primarily for their effects on the development of resistance in plants during the growth period prior to inoculation. However, in many cases it was necessary to maintain the varied conditions during the periods following inoculation. In addition to obtaining data which might assist in an interpretation of the effects of certain conditions on fire-blight resistance in plants under orchard conditions, it was thought that such a study was one of the first steps in an attempt to adduce evidence on the nature of fire-blight resistance.

Potted Northwestern Greening (resistant), Wealthy, Fameuse, and Yellow Transparent (susceptible) apple varieties were used in these studies which were made under greenhouse conditions.

SOIL NUTRIENTS

The relation of soil nutrients to fire-blight resistance in apple was studied in a single preliminary experiment. Five trees of Northwestern Greening and five of Wealthy were potted in sand and the same number in a soil composed of 1 part sand and 4 parts well-rotted sod. Light applications of sodium nitrate were made, at approximately 1-week intervals, to the trees in the sand-sod soil. The drain holes in the pots containing sand were plugged to prevent quick drainage of water from the pots. The relative fire-blight resistance of the shoots which developed on the trees was tested when they were 50 days old. On an average, 7 shoots of each variety were employed in each test. The results (fig. 3) show that soil nutrients had a distinct effect on fire-blight resistance in the Northwestern Greening shoots. Blight lesions which averaged 37 percent of the shoot length developed on the plants in the sand-sod soil, while the lesions averaged

only 15 percent of the shoot length in the plants in sand, indicating a greater resistance in the plants growing in sand. Only a slight difference in resistance was found between the Wealthy trees growing in the two types of soil. It is probable that with an increase in age of the shoot a more striking difference in resistance would have been obtained. These results are in agreement with those obtained by other investigators (*4*, *25*).

SOIL TEMPERATURE

The effect of various soil temperatures on fire-blight resistance in apple shoots was noted in these studies. One-year-old trees of Fameuse apple were placed in soil in galvanized-iron cans 8 inches in diameter and 16 inches in height. A layer of ground cork was placed on the surface of the soil in each can. The moisture of the soil was, in all cases, maintained at 75 percent of saturation (*32*, *v. 1*, *p. 150*).

About 2 weeks after the trees were potted they were placed in a series of Wisconsin soil-temperature tanks (*13*) with a range of temperatures from 12° to 32° C., as shown in figure 4. Eight trees were

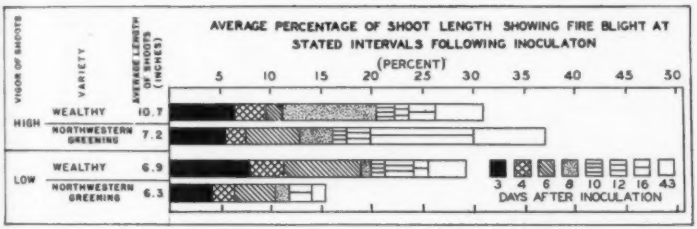
AVERAGE PERCENTAGE OF SHOOT LENGTH SHOWING FIRE BLIGHT AT STATED INTERVALS FOLLOWING INOCULATON
(PERCENT)
5 10 15 20 25 30 35 40 45 50
VIGOR OF SHOOTS
VARIETY
AVERAGE LENGTH OF SHOOTS (INCHES)
HIGH
WEALTHY 10.7
NORTHWESTERN GREENING 7.2
LOW
WEALTHY 6.9
NORTHWESTERN GREENING 6.3
3 4 6 8 10 12 16 43
DAYS AFTER INOCULATION

FIGURE 3.—Effect of soil nutrients on fire-blight resistance in Northwestern Greening and Wealthy apple shoots.

placed at each soil temperature. The average atmospheric temperature during the growth of the shoots was 21°; however, this average following inoculation of the shoots was approximately 24°. When the shoots were approximately 100 days old tests were made of their fire-blight resistance.

Results of these studies (fig. 4) indicate that soil temperature had a distinct but probably secondary effect on fire-blight resistance in the apple shoots employed. Shoots on trees at soil temperatures of 12° and 32° C., respectively, were the most resistant to fire blight; those at 20° and 24° were the most susceptible; and those at 16° and 28° fell in an intermediate class. No measurements were made of the fire-blight lesions beyond 12 days following inoculation. A positive relation between fire-blight resistance and lowered vigor in the shoots was indicated by the average lengths of the shoots at the different temperatures. It appears probable, therefore, that soil temperature influenced resistance and susceptibility because it affected certain growth conditions of the host which were related to resistance and susceptibility.

ATMOSPHERIC TEMPERATURE

The effects of various atmospheric temperatures on the development of fire blight in apple shoots were given attention by Brooks (*4*) and Miller (*16*). The data obtained by these authors indicated that the optimum temperature for fire-blight development was approxi-

mately 24° C. Miller (*16*) stated that at 16° disease development was so slow that practically no differences between the fire-blight resistance of Northwestern Greening and Wealthy apple shoots could be detected. However, at 24° and 28° Northwestern Greening was distinctly more resistant than Wealthy. Both of these writers worked with plants which were kept at the same temperature prior to inoculation and were placed at different temperatures following inoculation.

Further studies were made by the writer in which apple plants were grown at 16°, 20°, 24°, and 28° C., and were placed at 24° following inoculation. The different temperatures were obtained in a series of thermostatically controlled greenhouses. Northwestern Greening and Yellow Transparent were used. Ten trees of each variety were placed at each of the temperatures and were located so as to receive as nearly the same amount of illumination as possible. Two series of inoculations were made on shoots grown at the different temperatures. One was made on 21-day-old and one on 66-day-old shoots. On an average, eight shoots of each variety from each temperature was used in each inoculation series. This usually involved four trees. Immediately after the shoots were inoculated they were placed in a moist chamber (*14*) at 24° for 24 hours. Following the moisture treatment all of the trees were placed in a greenhouse at 24°, where they remained until final records were taken.

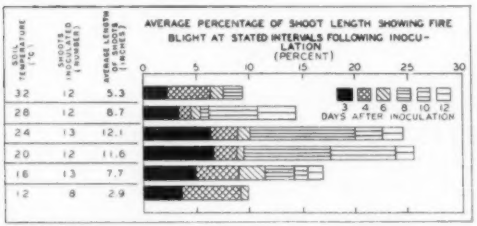
SOIL TEMPERATURE (°C.)
SHOOTS INOCULATED (NUMBER)
AVERAGE LENGTH OF SHOOTS (INCHES)
AVERAGE PERCENTAGE OF SHOOT LENGTH SHOWING FIRE BLIGHT AT STATED INTERVALS FOLLOWING INOCULATION (PERCENT)
0 5 10 15 20 25 30
3 4 6 8 10 12
DAYS AFTER INOCULATION
32 12 5.3
28 12 8.7
24 13 12.1
20 12 11.6
16 13 7.7
12 8 2.9

FIGURE 4.—Effect of various soil temperatures on fire-blight resistance in Fameuse apple shoots.

Combined results of the two inoculation series on both varieties show that the average percentages of the shoot lengths which blighted on the plants grown at 28°, 24°, 20°, and 16° C. were 29, 33, 41, and 54 percent, respectively. It appears, therefore, that 28° was the most favorable temperature for the development of resistance in the shoots; 16° was the least favorable; and 24° and 20° were intermediate. These preliminary results, when compared with those of Brooks (*4*) and Miller (*16*), indicate that the optimum temperature for fire-blight development in apple shoots is not the optimum temperature for the development of susceptibility of the host to the pathogene.

SOIL MOISTURE

The effect of soil moisture on fire-blight resistance and susceptibility in Northwestern Greening and Yellow Transparent apple varieties was studied (fig. 5, *B*, *C*, *D*, *E*). Twenty plants of each variety were grown at each of the following soil moistures: 40 to 50 percent and 80 to 90 percent of the moisture-holding capacity of the soil (*32*, *v. 1*, *p. 150*). The plants were grown in galvanized-iron cans 8 inches in diameter and 12 inches high. A layer of ground cork was placed on the surface of the soil in all of the containers to lessen the surface evaporation of the water. Water was added to the soil through aluminum tubes which were inserted in the soil when the trees were potted. Alternate notches were sawed in the tubes at approximately 1-inch intervals to facilitate an even distribution of water in the soil.

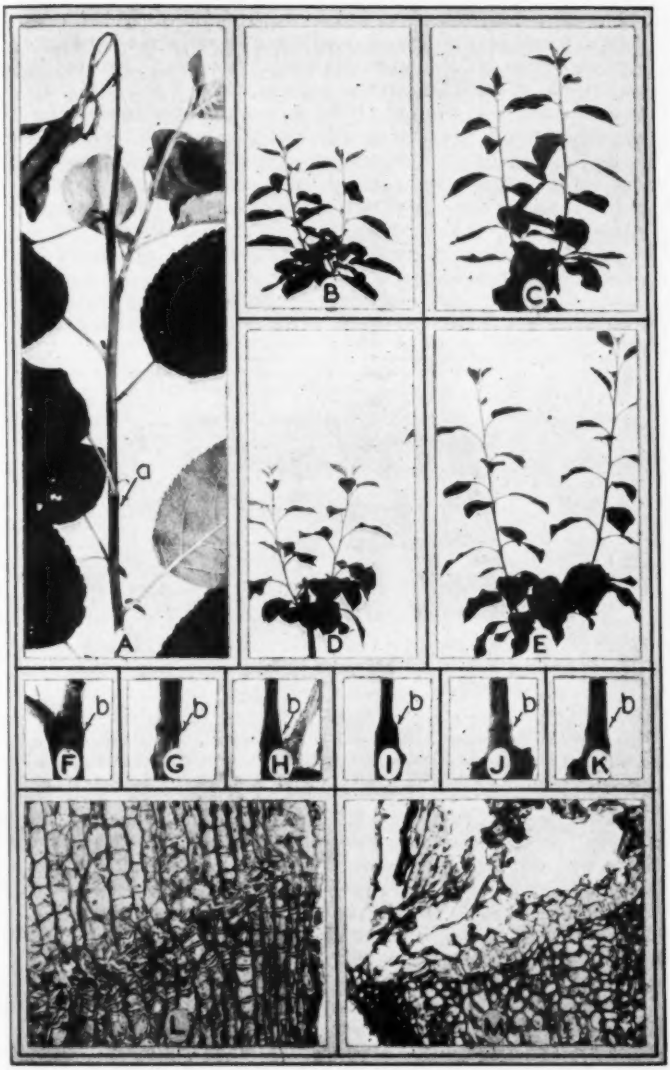

FIGURE 5.—*A*, Northwestern Greening apple shoot in which fire blight appeared at the base of a leaf petiole, *a*, at a considerable distance below the blight in the tip of the shoot; *B*, *C*, 37-day-old shoots of Yellow Transparent apple produced at 40 to 50 percent (*B*) and 80 to 90 percent (*C*) soil moistures, respectively; *D*, *E*, 37-day-old shoots of Northwestern Greening apple produced at 40 to 50 percent (*D*) and 80 to 90 percent (*E*) soil moistures, respectively; *F*, *G*, *H*, *I*, appearance of cork layers, *b*, between healthy and fire-blighted tissues in Northwestern Greening (*F*, *G*), Wealthy (*H*), and Yellow Transparent (*I*) apple shoots; *J*, *K*, appearance of cork layers, *b*, in Yellow Transparent apple shoots induced by flaming the apical one-fourth of the shoot (*J*) and by cutting off the apical one-fourth of the shoot (*K*); *L*, *M*, sections of cork layers between healthy and fire-blighted cortical tissues of Northwestern Greening apple shoots 12 days (*L*) and 43 days (*M*) after inoculation.

The interval at which water had to be added to the soil to maintain the different soil moistures varied with the amount of foliage on the plants, the weather conditions, and the percentage of the moisture-holding capacity of the soil desired. However, on an average, adjustments of the soil moistures were made at 3-day intervals.

Tests were made of the fire-blight resistance of plants at the different soil moistures when the shoots had reached the following ages: 26, 37, 50, and 89 days. On an average, 10.5 shoots of each variety at each soil moisture were used in each inoculation series. Usually 5 trees of a variety were involved. In all cases the plants were placed in a moist chamber (*14*) at 24° C. for 24 hours following inoculation.

The results of these studies (fig. 6) show that relatively low soil moistures were more favorable for increased fire-blight resistance in the plants than high soil moistures. Shoots on plants grown at 40 to 50 percent soil moisture were, with but minor exceptions, dis-

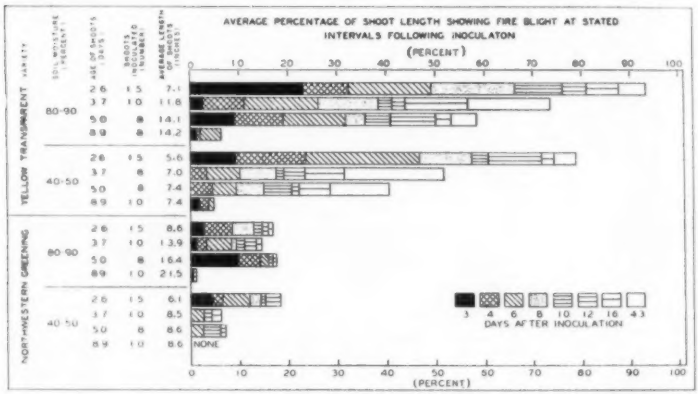

FIGURE 6.—Effect of various soil moistures on fire-blight resistance in Northwestern Greening and Yellow Transparent apple shoots of various ages.

tinctly more resistant to fire blight than shoots of the same age grown at 80 to 90 percent soil moisture. The effect of soil moisture on resistance was not pronounced in the older shoots, since an increase in the age of shoots at all soil moistures was accompanied by marked increases in resistance. In a given variety increased resistance was, with but minor exceptions, related to lowered vigor in the shoots, as indicated by their average lengths.

In two further experiments, each approximating the plan of the one described above, small trees and grafts of Northwestern Greening and Wealthy apple were grown at soil moistures ranging from 40 to 95 percent of saturation. The grafts were inoculated with culture A. The results obtained on the fire-blight resistance of these plants were in accord with those in figure 6.

ATMOSPHERIC HUMIDITY

Periods of relatively high atmospheric humidity, as indicated by the amount and duration of precipitation, have been reported by many investigators (*1*, *5*, *12*, *25*, *30*) as frequently accompanied or immediately followed by rapid development of fire blight in apple

and pear trees under orchard conditions. In the work of Gossard and Walton (*9*), Brooks (*4*), Miller (*16*), and Tullis (*28*), the importance of meteoric water in the dissemination of fire-blight inoculum was emphasized. In addition to the established importance of meteoric water as a disseminator of fire-blight inoculum in certain localities, the question is presented whether the high atmospheric humidity, which normally results from period of precipitation, may have an effect on the resistance and susceptibility of plants to the disease.

The relation of atmospheric humidity to fire-blight development in apple shoots was given attention by Brooks (*4*). He subjected potted Fameuse apple trees, following inoculation with *Erwinia amylovora*, to controlled relative atmospheric humidities of 50, 80, and 95 percent. In these studies the greatest amount of resistance to fire blight was shown by the shoots at 50 percent relative humidity.

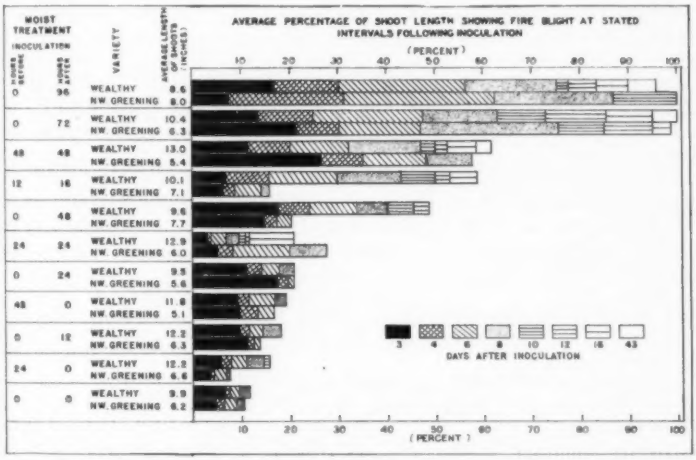

FIGURE 7.—Effect of atmospheric humidity on fire-blight resistance in Northwestern Greening and Wealthy apple shoots. The plants were treated in a moist chamber (*14*) at 24° C. Plants treated 12 hours before inoculation had moisture treatments from 1 to 5 p.m. for 4 days following inoculation.

Eighty percent was the most favorable for susceptibility, while 95 percent was slightly less favorable. Miller (*16*) described an experiment in which young Northwestern Greening apple shoots showed no more resistance to fire blight than comparable Wealthy shoots when placed in a moisture-saturated atmosphere at 26° C. following inoculation.

Further studies were made by the writer on the relation of atmospheric humidity to fire-blight resistance in apple shoots. Potted trees of Northwestern Greening and Wealthy were used. Accurate control of the soil moisture at which the trees were grown was not attempted in these experiments. Water was added to the soil in the pots at relatively short intervals to maintain a moisture condition which would promote good growth of the shoots. When the shoots were 50 days old they were inoculated with *Erwinia amylovora*. The trees were placed in a moist chamber (*14*) at 24° C. for varying periods immediately preceding and immediately following inoculation, as shown in figure 7. Artificial illumination from three 300-watt

Mazda lamps were given the plants from 8 a.m. until 6 p.m. After the moisture treatments the plants were placed in a greenhouse at 24°, where the relative humidity varied but averaged approximately 60 percent. Control plants were kept in this greenhouse. On an average five shoots of each variety were used in each moisture-treatment series. Usually three plants were used in each treatment.

Results of these studies (fig. 7) show that, on an average, the greatest fire-blight resistance in both varieties occurred in the plants which were not subjected to the high atmospheric humidity in the moist chamber. The fire-blight resistance of the shoots decreased, in most cases, as the duration of exposure to the high atmospheric humidity was increased following inoculation. Seventy-two-hour and ninety-six-hour exposures reduced resistance to the extent that practically all of the shoots blighted entirely. Twenty-four-hour and forty-eight-hour exposures before inoculation appeared to have but little effect on resistance. However, when such treatments were followed by comparable treatments after inoculation considerable reductions in fire-blight resistance took place in many cases. Moisture treatments from 1 to 5 p.m. for 4 days following inoculation greatly reduced fire-blight resistance in Wealthy shoots. However, similar treatments induced only a slight decrease in the resistance of Northwestern Greening.

This experiment was repeated twice on 28-day-old and 62-day-old shoots of Northwestern Greening and Yellow Transparent apple. Results similar to those in figure 7 were obtained. Increased exposures to the high humidity consistently caused increased blight susceptibility in the shoots.

THE RELATION OF CERTAIN INTERNAL HOST CONDITIONS TO RESISTANCE TO FIRE BLIGHT

Attempts to relate certain conditions within apple and pear shoots to resistance to fire blight have been made by a limited number of investigators. Arthur (*1*) did not note any positive relation between the moisture contents of shoots of various apple and pear varieties and their resistance to fire blight. However, among the different groups of pome fruits he did find a positive relation. He summarized his studies as follows (*1, p. 272*):

> There is found to be a rather constant ratio between the percentages of water in the branches of the several kinds of pome fruits, corresponding, to some extent, with their liability to blight, but this relation is not yet established for the several varieties of a single kind, e.g., pears * * *.

Hewitt (*10*), after making a series of chemical analyses of twigs of various apple varieties, reported a positive relation between fire-blight resistance and a low percentage of starch in the twigs. However, in this connection, Bachmann (*3*) did not observe any diminution in the amount of starch in the cells of blighting apple shoots; this indicates that starch content is probably not directly responsible for differences in resistance. Higgins (*11*) observed in stained sections of blighted apple and pear twigs deposits of a gummy substance in the border regions between the healthy and fire-blighted tissues. He suggested that the formation of the gummy substance might have a part in blight resistance.

The character of growth in apple and pear trees was associated with fire-blight resistance by some of the earliest observers of the disease (*2*). Trees which produced slow, woody growth were observed to be less liable to blight than those which produced rapid, succulent growth. These earlier observations were substantiated in many of the investigations which followed. Stewart (*25*) suggested that varietal differences in resistance to blight in pear were associated with the periods of growth activity of the plants. On July 8, 1912, he examined the conditions of growth of 12 pear varieties at Ithaca, N.Y. Three conditions of growth were observed: (1) Hard and woody, 4 varieties; (2) medium tender, 6; and (3) tender, 2. Artificial inoculations with *Erwinia amylovora* revealed that those varieties with hard and woody growth were very resistant to blight, those with tender growth were very susceptible, and those with medium-tender growth were intermediate.

In order to obtain more data relating to the nature of fire-blight resistance, studies were initiated on certain internal host conditions which appear to have a part in the resistance of plants to the disease. The host material used in these studies was taken from the apple trees employed in the experiments described in the foregoing sections of this paper.

MOISTURE CONTENT OF APPLE SHOOTS IN RELATION TO FIRE-BLIGHT RESISTANCE

Further studies of the moisture content of apple shoots in relation to fire-blight resistance seemed to be advisable in view of the suggestive results obtained by Arthur (*1*), and because of the almost universal belief that succulence of tissues is associated with susceptibility to the disease. Therefore, determinations were made of the moisture content of the terminal growth of orchard trees and shoots on potted plants in the greenhouse.

Studies on Orchard Trees

The moisture contents of the terminal shoots of 18-year-old trees of four apple varieties growing under orchard conditions were measured. The method of measuring the moisture content was as follows: Thirty terminal shoots, including the new growth and the previous year's wood, were collected from the desired varieties. These shoots were taken from representative regions of at least two trees of each variety. The collections were made between 4 and 5 p.m. The samples were wrapped in moist cheesecloth and placed in vascula so as to minimize loss of moisture while being taken to the laboratory. After the leaves were removed from the shoots each sample was divided into the following portions: (1) Apical half of new growth; (2) basal half of new growth; and (3) previous year's wood. These portions of the samples were weighed individually, after which they were placed in a drying oven at 105° C., where they remained until they came to a constant weight. When this condition was obtained the dry weight of each portion of the samples was taken. The moisture content of each portion of the samples was then computed and expressed as percentage of the fresh weight.

The moisture content of the terminal growth of 18-year-old Northwestern Greening, McIntosh, Wealthy, and Yellow Transparent apple trees was measured in 1931 on June 20, June 27, July 3, July 9, and

August 4. These studies were made on the same trees used in the inoculation experiments described on page 287. The moisture determinations (fig. 8, *A*, *B*, *C*, *D*) show that on June 20 the new growth on Northwestern Greening had a considerably lower percentage of moisture than comparable growth on Yellow Transparent. McIntosh and Wealthy were intermediate. From June 20 to July 9 a relatively

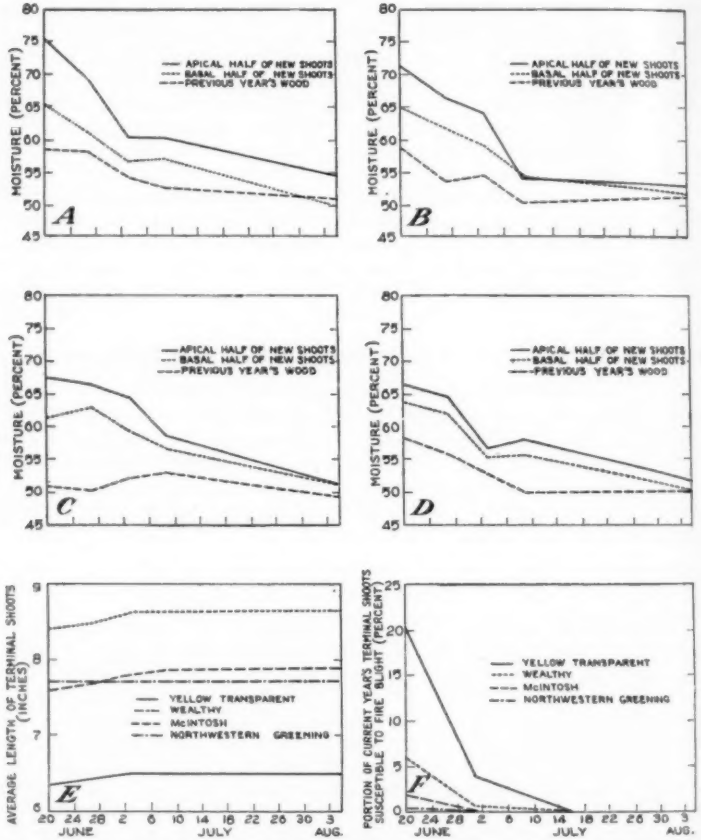

FIGURE 8.—Moisture content of the terminal growth of 18-year-old Yellow Transparent (*A*), Wealthy (*B*), McIntosh (*C*), and Northwestern Greening (*D*) apple varieties growing under orchard conditions; *E*, average length of the new terminal growth on the four varieties; *F*, relative fire-blight resistance of the new terminal growth on the four varieties.

rapid decrease in percentage of moisture took place in all of the varieties; on July 9 there were only slight differences between the moisture in the apical and in the basal halves of the new growth in most of the varieties. After July 9, varietal differences in moisture content tended to disappear. On August 4 a further drop in percentage of moisture was found in all of the varieties, and there were no significant differences among the different portions of shoots of the same variety, except in Yellow Transparent (fig. 8, *A*). In this

variety the percentage of moisture of the apical half of the new growth was noticeably higher than that of the basal half and that of the previous year's wood.

The fire-blight resistance of shoots comparable to those used in the moisture determinations was tested on June 20, July 1, July 16, and August 1, as described earlier (p. 287). Results of these tests (fig. 8, *F*) suggest a positive relation between increased fire-blight resistance and lowered percentage of moisture in the shoots. This relation seems to hold in comparisions of different varieties at any given interval, and in comparisons of tests on a given variety at progressive intervals during the season. This subject was investigated further with material grown in the greenhouse.

Studies on Plants Growing Under Greenhouse Conditions

The moisture content of twigs was varied by regulating the growing conditions for trees in soil cans in the greenhouse. Two lots each of Northwestern Greening and of Yellow Transparent apple trees were grown at 40 to 50 percent and 80 to 90 percent of the moisture-holding capacity of the soil, respectively. Measurements were made of the percentages of moisture in 26-, 37-, 50-, and 89-day-old shoots produced under these conditions. The method for measuring the percentage of moisture in the shoots was the same as that employed in the studies on orchard trees, as previously described, except that only 10 shoots were used in each group and the samples of shoots were divided into the following portions: (1) Apical third, (2) middle third, and (3) basal third.

These measurements (fig. 9, *A*, *B*, *C*, *D*) show that substantial differences in moisture content of the shoots were found between the two varieties and between shoots of the same variety grown at different soil moistures.

In the trees grown at 80 to 90 percent soil moisture, the apical two-thirds of the 26-day-old shoots of Yellow Transparent had a moisture content of 80 percent, and the basal third a moisture content of 77 percent; while the apical two-thirds of comparable Northwestern Greening shoots had a moisture content of approximately 77 percent, and the basal third a moisture content of 73 percent (fig. 9, *A*, *C*). With an increase in age of the shoots a drop in percentage of moisture took place in both of the varieties, but the moisture content of the apical third of the Yellow Transparent shoots remained approximately 5 percent higher than that of the comparable region of Northwestern Greening shoots. There was a marked tendency in Northwestern Greening for the percentage of moisture to become equal throughout the shoots, but this tendency was not pronounced in Yellow Transparent. In the 89-day-old shoots of Northwestern Greening no substantial differences in moisture content occurred among different regions of the shoots. However, in Yellow Transparent the apical third of the shoots had a moisture content approximately 5 percent higher than that of the middle third and approximately 8 percent higher than that of the basal third.

In the trees at 40 to 50 percent soil moisture no great differences in percentages of moisture were found among different regions of shoots of the same variety (fig. 9, *B*, *D*). In Yellow Transparent the moisture content of the entire shoots closely paralleled that of the basal third

of shoots of the same variety at 80 to 90 percent soil moisture. In Northwestern Greening the shoots at 40 to 50 percent soil moisture had a moisture content which was, on an average, about 5 percent lower than that of comparable Yellow Transparent shoots. Likewise, the decrease in moisture content with age appeared to be more rapid in Northwestern Greening than in Yellow Transparent.

Tests were made of the relative fire-blight resistance of shoots comparable to those used in the moisture determinations, as previ-

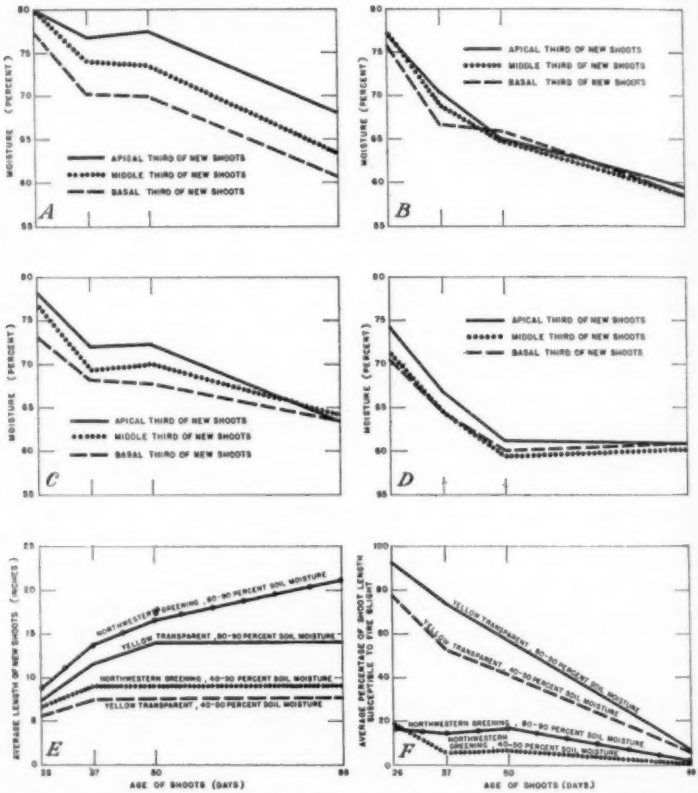

FIGURE 9.—Moisture content of shoots of Yellow Transparent grown at 80 to 90 percent (*A*), and at 40 to 50 percent soil moisture (*B*), and of Northwestern Greening at 80 to 90 percent (*C*) and 40 to 50 percent soil moisture (*D*); *E*, average length of shoots at the different soil moistures; *F*, relative fire-blight resistance of shoots at the different soil moistures.

ously described (p. 292). These experiments (fig. 9, *E*, *F*) show that, in general, a direct relation was found between a low percentage of moisture in the shoots and resistance to fire blight. The Northwestern Greening shoots produced at 40 to 50 percent soil moisture had the lowest percentage of moisture and were the most resistant to fire blight, while the Yellow Transparent shoots grown at 80 to 90 percent soil moisture had the highest and were the least resistant to blight. The Yellow Transparent shoots grown at 40 to 50 percent soil mois-

ture and the Northwestern Greening shoots grown at 80 to 90 percent soil moisture were intermediate with respect to percentage of moisture and resistance to blight.

CORKING OFF OF FIRE-BLIGHT LESIONS IN RELATION TO FIRE-BLIGHT RESISTANCE

The formation of cork layers which appear as barriers between the healthy and fire-blighted tissues in both apple and pear varieties has been noted by several investigators, including Miller (*16*), Tullis (*28*), and Rosen (*23*). Sometimes these cork layers appeared as much as 2 cm or more below the visible fire-blight lesion. Fire-blight cankers which are delimited by cork layers or fissures are generally believed to overwinter the fire-blight pathogene less frequently than those not delimited (*16, 28, 30*). Extension of the margins of delimited cankers has been reported to occur less frequently than of those not delimited (*4, 16, 23, 30*). Further studies were made by the writer on the relation of cork layers to fire-blight resistance in apple shoots.

Separation of the healthy and fire-blighted tissues by cork layers in the plants employed in the studies described in the foregoing sections, commonly occurred during the late stage of fire-blight development in the shoots (fig. 5, *F, G, H*). Paraffined sections were made of shoots in which the cork layers were in various stages of development. These studies revealed that in a given shoot the cork layer commonly involved the entire circumference of the shoot, and extended inwardly through the cortex, and apparently through the phloem and cambium (fig. 5, *L, M*); but a well-defined cork layer across the xylem and pith was not observed.

The location of the fire-blight pathogene in relation to the cork layers in Northwestern Greening and Wealthy apple shoots was studied in two ways: (1) By examination of stained sections made from various regions of shoots, and (2) by attempted isolations of the pathogene from tissues taken from various regions.

Material for sectioning was taken from the following regions of artificially inoculated Northwestern Greening and Wealthy apple shoots: (1) A portion of the shoot taken from a point about 3 cm from the cork layer toward the apical end of the shoot, (2) a portion of the shoot including the cork layer, and (3) a portion of the shoot taken from a point from 3 to 10 cm from the cork layer toward the basal end of the shoot. These tissues were killed and fixed in formalin-acetic alcohol, dehydrated in alcohol, placed in chloroform, and finally embedded in paraffin. Sections were made with a rotary microtome and stained with safranine followed by fast green. Microscopic examinations were made to determine the location of the fire-blight pathogene in the tissues.

Studies, as described above, were completed of 8 shoots from which the fixations were made 43 days following inoculation. Three of the shoots were Northwestern Greening and five were Wealthy. Toward the apical ends of the shoots from the cork layers, the fire-blight pathogene was found in the cortical, phloem, and cambial tissues of all shoots; and in the xylem and pith of 4. Toward the basal ends of the shoots from the cork layers the pathogen was found in the pith of 1 shoot; in the xylem of 2; but it was not found in the cortical, phloem, or cambial tissue of this region. These findings are in agreement with those of Rosen (*23*), and others.

Attempts were made to isolate the fire-blight pathogene from tissues taken from the following regions of artificially inoculated shoots: (1) A cross section of the shoot taken from a point about 1 cm from the cork layer toward the apical end of the shoot; (2) a small portion of the shoot including only the cortex, phloem, and fragments of the cambium taken from a point about 1 cm from the cork layer toward the basal end of the shoot; and (3) a portion of the xylem and pith taken from various points up to 10 cm from the cork layers toward the basal end of the shoot. After the portions of tissues were removed from the shoots they were surface sterilized in mercuric chloride and washed through 2 changes of sterile distilled water. Each portion of tissue from each shoot was then macerated in sterile distilled water in a Petri dish, and a series of dilution plates were made. When bacterial growth was obtained in a given series of plates, inoculations were made into shoots of potted Wealthy apple plants in the greenhouse to test the pathogenicity of the organism.

Isolations were attempted from 19 shoots, about half of which were Northwestern Greening and about half Yellow Transparent, approximately 43 days after the shoots were inoculated. The fire-blight pathogene was isolated toward the apical end of 18 shoots from the cork layers; from the xylem and pith toward the basal end of 10 shoots at various points from 3 to 10 cm from the cork layers; but not from the cortical, phloem, or cambial tissues of the base of any shoot.

The question arises, in view of these results, whether the cork layers and the xylem are barriers against the migration of the blight pathogene toward healthy cortical, phloem, and cambial tissues of infected apple shoots.

Observations were made to determine whether advance of the margins of any of the fire-blight lesions occurred after they had corked off in the apple shoots used in the foregoing experiments. The time at which the observations were made varied from 25 to 60 days after the formation of cork layers and averaged approximately 50 days. Advance of the margins of lesions after signs of corking-off had appeared was observed in 12 out of 223 Yellow Transparent shoots; 1 out of 84 Wealthy; none out of 32 McIntosh; and none out of 226 Northwestern Greening. Advance of the margins of the lesions eventually stopped in all of the shoots, and cork formation occurred. This usually happened at a relatively short distance below the point at which cork formation first appeared. Whether the pathogene migrated through the cork layers or whether the initial cork layers did not involve the entire circumference of the shoots in which extension of the lesions occurred was not determined. These observations indicate that, under the given conditions, advance of the margins of fire-blight lesions which are corked off may be expected to occur in only a small percentage of cases. They suggest also that advances are more liable to occur in susceptible than in resistant varieties.

An attempt was made to determine whether cork layers formed in blighted apple shoots were barriers to further progress of the fire-blight pathogene in the cortex, phloem, and cambium. Northwestern Greening shoots in which the blight lesions had become delimited by cork layers were used. Several trials were conducted on comparable shoots. (1) The cork layer on each of 5 shoots was broken by cutting through it at 4 or 5 points with a surface-sterilized scalpel within an

area of approximately 3 square centimeters. As mentioned earlier, a small amount of apparently healthy tissue often remained between the cork layer and the fire-blight lesion. Each shoot was then inoculated with *Erwinia amylovora*, at a point about 5 mm from the break in the cork layer toward the apical end. (2) The cork layers of four shoots were broken, but the shoots were not inoculated. (3) Each of five shoots was inoculated at a point about 5 mm from the cork layer toward the apical end of the shoot, but the cork layer was not broken. (4) Five shoots were inoculated at a point about 5 mm from the cork layers toward the basal ends of the shoots, but the cork layers were not broken. (5) On seven shoots the cork layers were not broken, and no inoculations were made. (6) Four shoots, comparable to those used in the foregoing treatments, except that no cork layers had formed in them, were used as further controls. No inoculations were made on these shoots.

The blade of the scalpel used in breaking the cork layers was surface sterilized after each insertion by being dipped in alcohol and passed through a flame. These precautions were taken to minimize the chances of carrying the pathogene through the region of the cork layer on the blade of the scalpel.

After these treatments the trees were placed in a moist chamber (*14*) at 24° C. for 72 hours. They were illuminated by three 300-watt Mazda lamps 8 hours each day they were in the moist chamber. After the moisture treatments the plants were placed in a greenhouse at 24° C. Records were made of the shoots 14 days after the experiments were begun to determine whether advance of the margins of any of the blight lesions had occurred.

TABLE 1.—*Barrier effect of cork layers between healthy and fire-blighted tissues in Northwestern Greening apple shoots against the passage of Erwinia amylovora*

Treatment of shoots	Shoots employed	Average length of shoots	Average length of fire-blight lesions		Lesions which showed advance after moisture treatment
			Before moisture treatment [1]	10 days after moisture treatment	
Cork layers not broken:	*Number*	*Inches*	*Inches*	*Inches*	*Number*
Second inoculation above layers	5	9.0	1.1	1.1	0
No second inoculation	7	8.3	2.0	2.0	0
Second inoculation below cork layers [2]	5	7.7	.7	2.6	5
Cork layers broken:					
Second inoculation above cork layers	5	8.7	1.6	2.4	2
No second inoculation	4	7.9	.5	.8	2
No cork layers:					
No second inoculation	4	9.0	1.8	4.2	4

[1] Treatment in moist chamber for 72 hours at 24° C.

[2] The increase in lesion length recorded for this treatment should be considered as the length of the new lesions, since the extension of blight apparently resulted from the second inoculations.

Extension of the margins of the fire-blight lesions did not occur in any of the shoots in which the cork layers were not artificially broken (table 1). Substantial extension took place, however, in shoots in which the cork layers were broken, regardless of whether the shoots were reinoculated with the blight pathogene at the beginning of the experiment. These preliminary data indicated that the cork layers acted as barriers to further invasion of the cortical, phloem, and cambial tissues by the fire-blight pathogene in the apple shoots employed.

As indicated in earlier experiments, cork layers do not in all cases prevent the extension of the margins of fire-blight lesions. However, the data, as a whole, strongly indicate that, under the stated conditions, the cork layers were barriers to further advance of the pathogene in the cortical, phloem, and cambial tissues in a very high percentage of the shoots.

Movement of the fire-blight pathogene from the xylem to the cambial, phloem, and cortical tissues toward the basal end of shoots from the cork layers apparently occurred in 7 shoots out of a total of approximately 800 which were observed. These shoots were on potted plants growing in the greenhouse. Two of the shoots were Northwestern Greening, 2 were Wealthy, and 3 were Yellow Transparent. In each of these shoots seeming movement of the pathogene from the xylem was indicated by the appearance of cortical blight at the base of a leaf petiole at some distance toward the basal end of the shoot from the cork layer (fig. 5, *A*). A connection between the blight in the tip of each shoot and that which appeared at the base of the leaf petiole was found in the form of one or more discolored strands of xylem. *Erwinia amylovora* was isolated from the discolored strands of xylem from each shoot. These results suggested, although they did not prove, that the blight pathogene emerged from the xylem at the base of the leaf petiole and that the organisms from this source produced the cortical blight at that point.

An attempt was made to determine whether the fire-blight pathogene, when artificially introduced into Northwestern Greening and McIntosh shoots, was able readily to move from the xylem and pith tissues and produce cortical blight. In order to introduce the pathogene into the xylem and pith of a given shoot, a small ellipsoidal portion of the cortical, phloem, and cambial tissues was removed from one side of the shoot at a point about midway between the tip and base. This opening was filled with paraffin, and the xylem and pith were inoculated with *Erwinia amylovora*, through the paraffin. Six shoots were so treated. Four were treated similarly, except that the shoots were not inoculated, to test the effect of paraffin on the tissues. In two shoots the ellipsoidal portions of tissues were removed, but the wounds were not filled with paraffin. The xylem and pith were inoculated. This was done to determine whether the paraffin was effective in preventing the introduction of the pathogene into the wounded cortical, phloem, and cambial tissues when the xylem and pith were inoculated. In four shoots the cortical tissues were inoculated, to test their susceptibility to the pathogene.

No cortical blight appeared on any of the shoots in which the pathogene was introduced directly into the xylem and pith, while in shoots the cortical tissues of which were inoculated, lesions averaging 1.2 inches in length developed. The other control plants showed (1) that the paraffin was effective in preventing the introduction of the pathogene into the wounded cortex, phloem, and cambium when inoculations of the xylem and pith were made; and (2) that the paraffin did not injure the tissues. At the end of 36 days after inoculation the shoots of which only the xylem and pith were inoculated were removed from the trees and split longitudinally at various angles. Discolored strands of xylem tissues were found to extend an average

of 1.9 inches in both directions from the points of inoculation in the shoots, whereas there was only a slight amount of discoloration in the pith. Isolations were attempted from discolored strands of xylem from 5 shoots, and the fire-blight pathogene was obtained from 4. These limited data, in accord with previous observations, suggest that under the given conditions, the fire-blight pathogene could not readily move from the xylem and pith of apple shoots and invade the cambial, phloem, and cortical tissues.

The time after inoculation required for the macroscopic appearance of cork layers between the healthy and fire-blighted tissues was determined by examining the shoots of the inoculated plants described in the foregoing sections, at definite intervals after inoculation (figs. 10 and 11). Records were made of the number of shoots showing macroscopic cork layers (fig. 5, *F*, *G*, *H*). The percentages of the total number of shoots in a given series of inoculations which showed cork layers were then computed for the different intervals. For simplification, these percentages were divided into the following classes: 0 (0 to 5), 10 (6 to 15), 20 (16 to 30), 40 (31 to 50), 60 (51 to 70), 80 (71 to 90), and 100 (91 to 100). Only the new terminal growth on the plants was considered. Consequently, when the blight lesions involved all of the new shoot growth and extended into the older wood, the lesions were recorded as not corked off. The fact that this occurred in only a relatively few cases made it advisable to give only the data concerning the new growth.

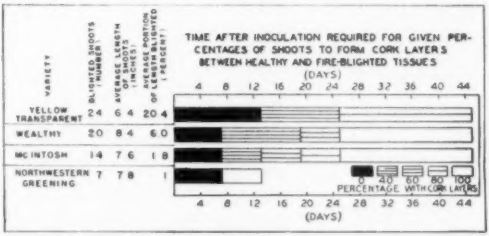
VARIETY
BLIGHTED SHOOTS (NUMBER)
AVERAGE LENGTH OF BLIGHTED SHOOT (INCHES)
AVERAGE PORTION OF SHOOT BLIGHTED (PERCENT)
YELLOW TRANSPARENT 24 6 4 20 4
WEALTHY 20 8 4 6 0
McINTOSH 14 7 6 1 8
NORTHWESTERN GREENING 7 7 8 1
TIME AFTER INOCULATION REQUIRED FOR GIVEN PERCENTAGES OF SHOOTS TO FORM CORK LAYERS BETWEEN HEALTHY AND FIRE-BLIGHTED TISSUES
(DAYS)
4 8 12 16 20 24 28 32 36 40 44
0 10 20 40 60 80 100
PERCENTAGE WITH CORK LAYERS
4 8 12 16 20 24 28 32 36 40 44
(DAYS)

FIGURE 10.—The time after inoculation required for the formation of cork layers between the healthy and fire-blighted tissues in shoots of 18-year-old apple trees of different varieties growing in the orchard, and the average percentages of the shoot lengths blighted 45 days after inoculation.

In the time required after inoculation for given percentages of apple shoots to form cork layers between the healthy and fire-blighted tissues, varietal differences were found. The corking-off of the lesions took place in a majority of the cases in a considerably shorter period of time in the resistant than in the susceptible varieties. For example, all of the blight lesions on the Northwestern Greening orchard shoots (fig. 10) were corked off at the end of 13 days following inoculation, while none of the Yellow Transparent shoots showed signs of corking-off at that time. Only 80 percent of the lesions on Yellow Transparent shoots had corked off at the end of 45 days following inoculation. McIntosh and Wealthy were in an intermediate class.

Results similar to those on orchard trees were obtained with potted plants in the greenhouse (fig. 11). In general, the fire-blight lesions on resistant varieties corked off in a shorter time after inoculation than those on susceptible varieties. For example, in the Northwestern Greening and Arkansas shoots all of the lesions were corked off at the end of 12 and 16 days, respectively, after inoculation, while in several varieties, including Yellow Transparent and Pewaukee, only about 80 percent of the lesions were corked off at the end of 43 days after inoculation. Many of the other varieties were in an intermediate class.

Low soil moistures were more favorable for the corking-off of fire-blight lesions on Northwestern Greening and Yellow Transparent

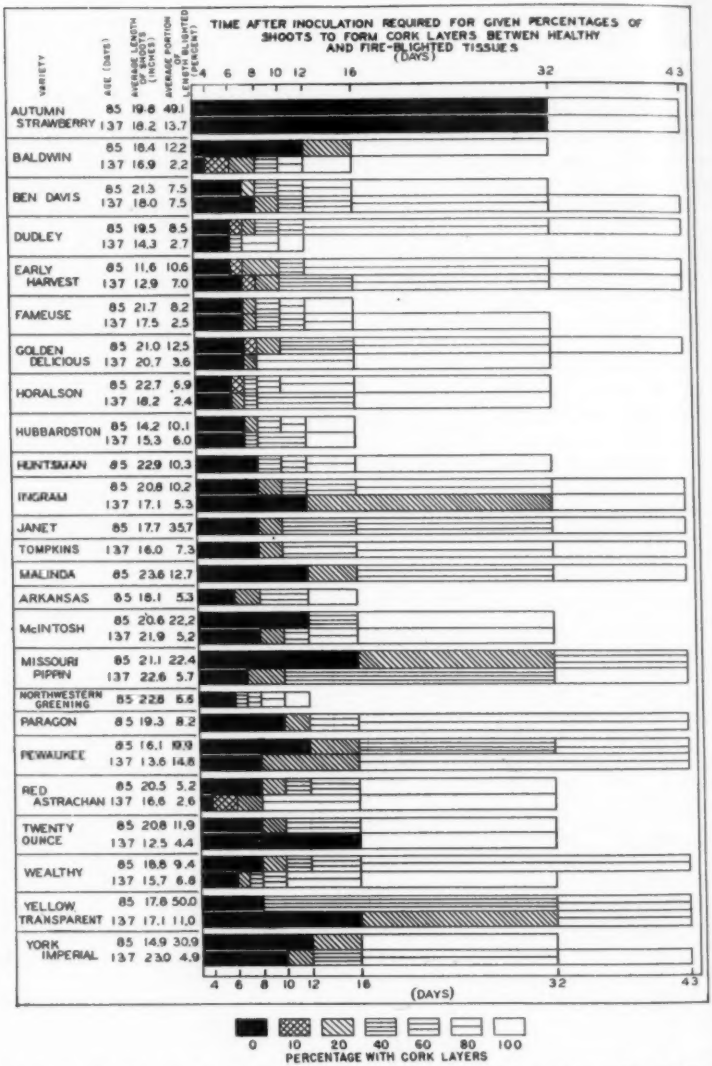
TIME AFTER INOCULATION REQUIRED FOR GIVEN PERCENTAGES OF SHOOTS TO FORM CORK LAYERS BETWEN HEALTHY AND FIRE-BLIGHTED TISSUES
(DAYS)
4 6 8 10 12 16 32 43
VARIETY
AGE (DAYS)
AVERAGE LENGTH OF SHOOTS (INCHES)
AVERAGE PORTION OF LENGTH BLIGHTED (PERCENT)
AUTUMN STRAWBERRY 85 19.8 49.1
137 18.2 13.7
BALDWIN 85 18.4 12.2
137 16.9 2.2
BEN DAVIS 85 21.3 7.5
137 18.0 7.5
DUDLEY 85 19.5 8.5
137 14.3 2.7
EARLY HARVEST 85 11.6 10.6
137 12.9 7.0
FAMEUSE 85 21.7 8.2
137 17.5 2.5
GOLDEN DELICIOUS 85 21.0 12.5
137 20.7 3.6
HORALSON 85 22.7 6.9
137 18.2 2.4
HUBBARDSTON 85 14.2 10.1
137 15.3 6.0
HUNTSMAN 85 22.9 10.3
INGRAM 85 20.8 10.2
137 17.1 5.3
JANET 85 17.7 35.7
TOMPKINS 137 16.0 7.3
MALINDA 85 23.6 12.7
ARKANSAS 85 18.1 5.3
McINTOSH 85 20.6 22.2
137 21.9 5.2
MISSOURI PIPPIN 85 21.1 22.4
137 22.6 5.7
NORTHWESTERN GREENING 85 22.8 5.6
PARAGON 85 19.3 8.2
PEWAUKEE 85 16.1 19.9
137 13.6 14.8
RED ASTRACHAN 85 20.5 5.2
137 16.6 2.6
TWENTY OUNCE 85 20.8 11.9
137 12.5 4.4
WEALTHY 85 18.8 9.4
137 15.7 6.8
YELLOW TRANSPARENT 85 17.8 50.0
137 17.1 11.0
YORK IMPERIAL 85 14.9 30.9
137 23.0 4.9
4 6 8 10 12 16 32 43
(DAYS)
0 10 20 40 60 80 100
PERCENTAGE WITH CORK LAYERS

FIGURE 11.—The time after inoculation required for the formation of cork layers between the healthy and fire-blighted tissues in shoots on potted plants of different apple varieties in the greenhouse, and the average percentages of the shoot lengths blighted 43 days after inoculation. (For further details of blight development in these shoots see fig. 2.)

apple shoots than high soil moistures (fig. 12). There was a positive correlation between cork formation and resistance to blight. This

experiment was repeated twice with small trees and grafts of Northwestern Greening and Wealthy. Results comparable to those shown in figure 12 were obtained.

Subjecting Northwestern Greening and Wealthy shoots to an atmospheric humidity approximating saturation for varying periods before and after inoculation caused considerable variation in the time after inoculation required for the corking-off of the fire-blight lesions (fig. 13). In general, the delay in the corking-off of the blight lesions was in proportion to the duration of the exposure of the plants to the high humidity following inoculation. All shoots which were not subjected to the high humidity had formed cork layers at the end of 10 days after inoculation. This did not occur at that time in any of the experimental groups which were given a moisture treatment. The formation of cork layers was prevented in the new shoot growth of practically all of the trees given 72-hour and 96-hour moisture

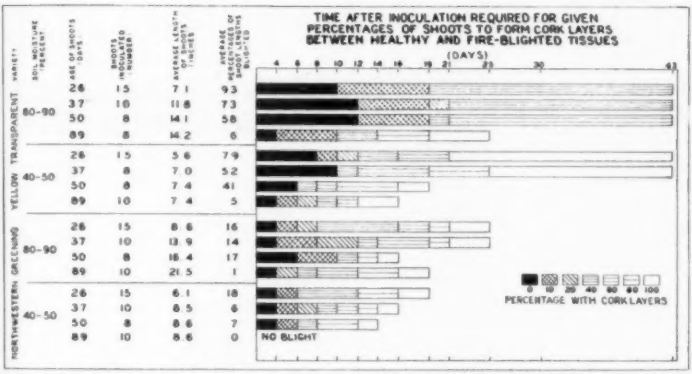
TIME AFTER INOCULATION REQUIRED FOR GIVEN PERCENTAGES OF SHOOTS TO FORM CORK LAYERS BETWEEN HEALTHY AND FIRE-BLIGHTED TISSUES
(DAYS)
4 6 8 10 12 14 16 18 21 23 30 43
VARIETY
SOIL MOISTURE PERCENT
AGE OF SHOOTS DAYS
SHOOTS INOCULATED NUMBER
AVERAGE LENGTH OF BLIGHTED SHOOTS INCHES
AVERAGE PERCENTAGES OF SHOOTS BLIGHTED
YELLOW TRANSPARENT
80-90
26 15 7.1 93
37 10 11.8 73
50 8 14.1 58
89 8 14.2 6
40-50
26 15 5.6 79
37 8 7.0 52
50 8 7.4 41
89 10 7.4 5
NORTHWESTERN GREENING
80-90
26 15 8.6 16
37 10 13.9 14
50 8 18.4 17
89 10 21.5 1
40-50
26 15 6.1 18
37 10 8.5 6
50 8 8.6 7
89 10 8.6 0
NO BLIGHT
0 10 20 40 60 80 100
PERCENTAGE WITH CORK LAYERS

FIGURE 12.—Effect of various soil moistures on the time after inoculation required for the formation of cork layers between the healthy and fire-blighted tissues of Northwestern Greening and Yellow Transparent apple shoots of various ages, and the average percentages of the shoot lengths blighted 43 days after inoculation. (For further details of blight development in these shoots see fig. 6.)

treatments following inoculation. Treatments for shorter periods delayed the corking-off of the lesions, roughly in proportion to the duration of the treatments. In practically all cases the blight lesions corked off in a shorter time on Northwestern Greening than on Wealthy shoots.

This experiment was repeated twice, with Northwestern Greening and Yellow Transparent. Results comparable to those shown in figure 13 were obtained. Increases in duration of exposure of plants to the high humidity again were followed by increases in the time after inoculation required for cork layer formation. Also the corking-off of the lesions took place in most of the experimental groups in a shorter time in Northwestern Greening than in Yellow Transparent.

Further studies were made of the time required for the formation of cork layers in Northwestern Greening and Yellow Transparent. Shoots of each variety were treated as follows: (1) Apical one-fourth killed by flaming; (2) inoculated at the apical end with *Erwinia amylovora;* and (3) apical one-fourth cut off. Six shoots of each variety were used in each treatment. The shoots were observed for

macroscopic signs of cork layers (fig. 5, *I*, *J*, *K*) at definite intervals following treatment. The results of these studies (fig. 14) show that the time required for formation of cork layers varied both with the variety and with the treatment. In all cases formation of cork layers took place in a shorter time in Northwestern Greening than in Yellow Transparent. In both varieties the formation of cork layers occurred soonest in the shoots which were flamed, and last in the shoots from which the apical portions were cut off. The blighted shoots were intermediate. These preliminary data, with those previously described, suggest that the formation of cork layers is more readily induced in Northwestern Greening (resistant) than in Yellow Transparent (susceptible) apple shoots.

The specific conditions in the host tissues which had a part in the corking-off of fire-blight lesions received little attention. However,

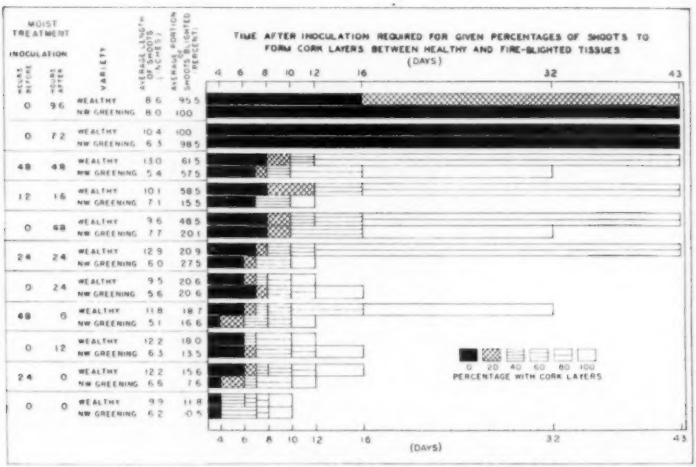
MOIST TREATMENT
INOCULATION
HOURS BEFORE
HOURS AFTER
VARIETY
AVERAGE LENGTH OF SHOOTS (INCHES)
AVERAGE PORTION SHOOTS BLIGHTED PERCENT
TIME AFTER INOCULATION REQUIRED FOR GIVEN PERCENTAGES OF SHOOTS TO FORM CORK LAYERS BETWEEN HEALTHY AND FIRE-BLIGHTED TISSUES
(DAYS)
4 6 8 10 12 16 32 43
0 96 WEALTHY 8 6 95 5
NW GREENING 8 0 100
0 72 WEALTHY 10 4 100
NW GREENING 6 3 98 5
48 48 WEALTHY 13 0 61 5
NW GREENING 5 4 57 5
12 16 WEALTHY 10 1 58 5
NW GREENING 7 1 15 5
0 48 WEALTHY 9 6 48 5
NW GREENING 7 7 20 1
24 24 WEALTHY 12 9 20 9
NW GREENING 6 0 27 5
0 24 WEALTHY 9 5 20 6
NW GREENING 5 6 20 6
48 0 WEALTHY 11 8 18 7
NW GREENING 5 1 16 6
0 12 WEALTHY 12 2 18 0
NW GREENING 6 3 13 5
24 0 WEALTHY 12 2 15 6
NW GREENING 6 6 7 6
0 0 WEALTHY 9 9 11 8
NW GREENING 6 2 0 5
0 20 40 60 80 100
PERCENTAGE WITH CORK LAYERS
4 6 8 10 12 16 32 43
(DAYS)

FIGURE 13.—Effect of high atmospheric humidity on the time after inoculation required for the formation of cork layers between the healthy and fire-blighted tissues in Northwestern Greening and Wealthy apple shoots, treated in a moist chamber at 24° C. Average percentages of shoot lengths blighted 43 days after inoculation are shown. Plants treated 12 hours before inoculation had moisture treatments from 1 to 5 p.m. on the 4 days following inoculation.

examinations of stained sections of blighted shoots, including the regions of the cork layers, indicated that in a given shoot one of the first steps was the suberization of the walls of a layer of cortical and phloem cells at some distance (up to 2 cm) toward the basal end of the shoot from the pathogene in the tissues. This step was followed by divisions in a layer of cells adjacent to the suberized layer toward the basal end of the shoot. This dividing layer of cells was interpreted to be of the nature of a cork cambium, and the resulting tissue has been referred to in the foregoing sections of this paper as a cork layer. These preliminary observations indicate that the series of reactions which lead to the formation of cork layers in blighting apple shoots are essentially similar to those observed by Priestly and Woffenden (*20*) and Priestly and Swingle (*19*) in their studies on the general problem of cork formation in plants.

It should be emphasized that the studies reported in the present paper were made primarily on artificially inoculated plants under greenhouse conditions. More work needs to be done on this problem, particularly on the relation of cork layers to fire-blight resistance in orchard trees.

DISCUSSION

The conclusion that characteristic differences in fire-blight resistance exist among rosaceous species and varieties seems to be justified by the data already given (figs. 1 and 2). Of the groups of species studied, those belonging to the genus *Malus* appeared to be the least resistant to the disease. Of the varieties of *M. malus* studied most intensively, Northwestern Greening was the most resistant and Yellow Transparent the most susceptible. McIntosh and Wealthy were intermediate, with McIntosh characteristically the more resistant. These conclusions with reference to apple varieties are in accord with those of various authors, including Stewart (*25*), Brooks (*4*), and Miller (*16*).

The degree of resistance or susceptibility which a given apple variety exhibited appeared to be related to two major factors: (1) The

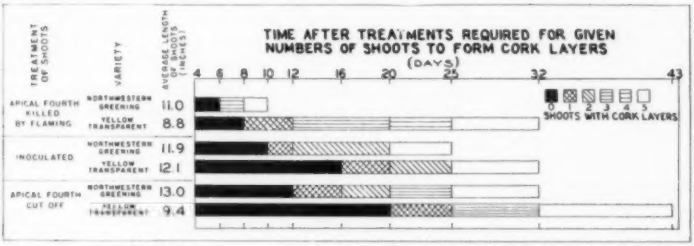

FIGURE 14.—Cork formation in Northwestern Greening and Yellow Transparent apple varieties after the specified treatments; six shoots of each variety were used in each treatment.

age of the shoots, or more specifically the physiological and morphological maturity of the tissues; and (2) the environmental conditions to which the plants were subjected. The relative importance of these two factors in relation to increased or decreased fire-blight resistance cannot be accurately estimated from the data at hand. However, certain interrelations of the two factors appear probable. It would seem that low soil nutrients, low and high soil temperatures, and high atmospheric temperatures contribute, in part at least, to increased resistance because they favor an early cessation of growth, which indicates early maturation of tissues. The intermediate or opposite conditions would favor increased susceptibility because they do not favor early maturation of tissues and early cessation of growth. Apparently similar relations have been described for certain other diseases, particularly the rusts and smuts of certain cereals (*15*, *21*).

High atmospheric humidity appeared to foster a condition of increased susceptibility which tended to minimize expression of varietal differences in resistance and to overcome resistance resulting from increased maturation of tissues (fig. 7). The nature of this increased susceptibility was not investigated. However, the writer is in agreement with the suggestion of Miller (*16*) that moisture conditions in

the intercellular spaces of host tissues play a part in causing differences in resistance and susceptibility in plants at different asmospheric humidities.

Soil moisture probably affects both the rate of maturation of tissues and moisture conditions in the intercellular spaces.

In this and certain other phases of the work, it appears that further trials with larger numbers conducted under a variety of conditions might seem desirable. A variety of limitations have thus far made such additional work impossible. However, it should be noted that many of the experiments described are closely interrelated and that they mutually support one another. The possibility that the differences in resistance among the plants resulted from variations in bacterial strains was avoided by using the same culture in parallel experiments.

Preliminary data showed a rather consistent direct relation between lowered percentages of moisture in apple shoots and resistance to fire blight (figs. 8 and 9). These data seem in accord with the opinion prevalent among plant pathologists and horticulturists that increased resistance to fire blight is associated, in part at least, with decreased succulence of tissues. Further, these data seem in accord with those showing a direct relation between increased age of shoots and increased fire-blight resistance (figs. 2, 6, 8, and 9). Although moisture content is important, it is doubtless only one factor in this complex relation.

The positive relation found between a decrease in the time after inoculation required for the corking-off of fire-blight lesions and increased resistance to fire blight appeared to be a significant one (figs. 11, 12, and 13). However, the data indicate that the primary importance of cork layers lies in the prevention of further migration of the pathogene after the lesions are corked off, although there was some evidence that corky tissues formed more rapidly in resistant than in susceptible varieties (fig. 14).

Relations strikingly similar to those just described for fire blight were found by Conant (*6*) in his studies on the resistance of tobacco varieties to *Thielavia basicola*, and by Tisdsale (*27*) in studies on the resistance of flax to *Fusarium lini*. Both of these writers believed that the high resistance of certain varieties was due, in part at least, to their ability to form cork layers in advance of the fungal hyphae. In many cases the fungal hyphae apparently were unable to penetrate the cork tissues.

There appears to be, therefore, considerable justification for concluding that resistance to fire blight in apple, and probably in other hosts of *Erwinia amylovora*, is related to three factors: (1) The inherent characters of the variety concerned; (2) the physiological and morphological maturity of the tissues; and (3) the environmental conditions to which the plants are subjected. However, the exact nature of the factors within the host tissues which are directly responsible for variations in resistance and susceptibility remain to be investigated.

SUMMARY

Wide differences in fire-blight resistance were found among 31 rosaceous species of the following genera: *Amelanchier*, *Aronia*, *Cotoneaster*, *Crataegus*, *Malus*, *Pyrus*, and *Sorbus*. Differences in resistance were more marked between species of *Crataegus* than between different genera. Of those studied, the plants in the genus *Malus* were the least resistant of all to the disease.

Wide differences in resistance to fire blight were found among 25 apple varieties. These differences took the same general order in the varieties studied under both greenhouse and orchard conditions. Of the 4 varieties studied most intensively, Northwestern Greening was consistently the most resistant and Yellow Transparent the least. McIntosh and Wealthy were intermediate, with some variation in their relative resistance.

An increase in age of apple shoots under both orchard and average greenhouse conditions was found to be accompanied by increased resistance to blight. This increase in resistance appeared to take place most rapidly in Northwestern Greening and slowest in Yellow Transparent. McIntosh and Wealthy were intermediate.

Low soil nutrients were found to be more favorable for the development of fire-blight resistance in Northwestern Greening and Wealthy apple shoots than high soil nutrients.

Soil temperatures of 12° and 32° C. were more favorable for the development of fire-blight resistance in Fameuse apple shoots than the intermediate temperatures 16°, 20°, 24°, and 28°; 20° and 24° were the least favorable for development of resistance in the shoots. There was a positive relation between resistance and lowered vigor in the shoots.

An atmospheric temperature of 28° C. in the growth period of plants prior to inoculation was the most favorable of the four temperatures studied, for the development of fire-blight resistance in Northwestern Greening and Yellow Transparent shoots. Sixteen degrees was the least favorable for the development of resistance, and 20° and 24° were intermediate.

Relatively low soil moistures were found to be more favorable for the development of fire-blight resistance in Northwestern Greening, Wealthy, and Yellow Transparent shoots than relatively high soil moistures.

Subjecting Northwestern Greening and Wealthy apple trees to an atmosphere approximately saturated with moisture, at 24° C., greatly altered their resistance to fire blight. Resistance to the disease decreased, in most cases, as the duration of the exposure of the plants after inoculation to the high humidity was increased. In most cases the new shoots blighted entirely on trees subjected to the high humidity for 72 and 96 hours following inoculation.

A positive relation was found between a low percentage of moisture and fire-blight resistance in the new terminal growth of Northwestern Greening, McIntosh, Wealthy, and Yellow Transparent trees.

Separation of the healthy from the fire-blighted tissues by cork layers commonly occurred during the late stage of blight development in the plants employed in these studies. Examinations of paraffined sections of shoots revealed that in a given shoot the cork layer commonly involved the entire circumference of the shoot and extended inward through the cortex and apparently through the phloem and cambium; but a well-defined cork layer inside the cambium was not observed.

Examinations of stained sections of apple shoots and attempts to isolate the fire-blight pathogene from various regions of blighted shoots revealed that the pathogene was commonly present in all tissues toward the apical ends of the shoots from the cork layers, and

in the xylem and pith at considerable distances toward the basal ends of the shoots from the cork layers; but it was not found in the cortex, phloem, or cambium of the basal ends.

Observations and experiments are described which indicate that once the fire-blight lesions are corked off, the cork layers and the xylem commonly serve as relatively effective barriers against further invasion of the cortical, phloem, and cambial tissues by the pathogene.

The time after inoculation required for the corking-off of fire-blight lesions varied in different varieties of apple. In general, the more resistant the variety to fire blight the shorter the time after inoculation required for the corking-off of the blight lesions.

Low soil moistures favored the corking-off of fire-blight lesions.

Subjecting plants to a high atmospheric humidity following inoculation delayed the corking-off of blight lesions.

LITERATURE CITED

(1) Arthur, J. C.
1887. pear blight. micrococcus amylovorus b. N.Y. State Agr. Expt. Sta. Rept. (1886) 5: 259–273, illus.

(2) ———
1887. important articles on pear blight. N.Y. State Agr. Expt. Sta. Rept. (1886) 5: 283–296.

(3) Bachmann, F. M.
1913. the migration of bacillus amylovorus in the host tissues. Phytopathology 3: 3–14, illus.

(4) Brooks, A. N.
1926. studies of the epidemiology and control of fire blight of apple. Phytopathology 16: 665–696, illus.

(5) Burrill, T. J.
1879. fire blight. Ill. State Hort. Soc. Trans. (n.s.) (1878) 12: 79–80.

(6) Conant, G. H.
1927. histological studies of resistance in tobacco to thielavia basicola. Amer. Jour. Bot. 14: 457–480, illus.

(7) Crandall, C. S.
1898. blight and other plant diseases. Colo. Agr. Expt. Sta. Bull. 41, 21 pp.

(8) Elliott, C.
1930. manual of bacterial plant pathogens. 349 pp. Baltimore.

(9) Gossard, H. A., and Walton, R. C.
1922. dissemination of fire blight. Ohio Agr. Expt. Sta. Bull. 357, pp. 83–126, illus.

(10) Hewitt, J. L.
1913. twig blight and blossom blight of the apple. Ark. Agr. Expt. Sta. Bull. 113, pp. 493–505.

(11) Higgins, B. B.
1919. gum formation with special reference to cankers and decays of woody plants. Ga. Agr. Expt. Sta. Bull. 127, pp. 23–59, illus.

(12) Jones, D. H.
1909. bacterial blight of apple, pear, and quince trees. Ontario Dept. Agr. Bull. 176, 63 pp., illus.

(13) Jones, L. R., Johnson, J., and Dickson, J. G.
1926. wisconsin studies upon the relation of soil temperature to plant diseases. Wis. Agr. Expt. Sta. Research Bull. 71, 144 p., illus.

(14) Keitt, G. W., and Jones, L. K.
1926. studies of the epidemiology and control of apple scab. Wis. Agr. Expt. Sta. Research Bull. 73, 104 pp., illus.

(15) Kyle, C. H.
1930. relation between the vigor of the corn plant and its susceptibility to smut (ustilago zeae). Jour. Agr. Research 41: 221–231.

(16) MILLER, P. W.
1929. STUDIES OF FIRE BLIGHT OF APPLE IN WISCONSIN. Jour. Agr. Research 39: 579–621, illus.
(17) NIXON, E. L.
1927. THE MIGRATION OF BACILLUS AMYLOVORUS IN APPLE TISSUE AND ITS EFFECT ON THE HOST CELLS. Pa. Agr. Expt. Sta. Tech. Bull. 212, 16 pp., illus.
(18) PIERSTORFF, A. L.
1931. STUDIES ON THE FIRE-BLIGHT ORGANISM, BACILLUS AMYLOVORUS. N.Y. (Cornell) Agr. Expt. Sta. Mem. 136, 53 pp., illus.
(19) PRIESTLY, J. H., and SWINGLE, C. F.
1929. VEGETATIVE PROPAGATION FROM THE STANDPOINT OF PLANT ANATOMY. U.S. Dept. Agr. Tech. Bull. 151, 99 pp., illus.
(20) ——— and WOFFENDEN, L. M.
1922. PHYSIOLOGICAL STUDIES IN PLANT ANATOMY. V. CAUSAL FACTORS IN CORK FORMATION. New Phytol. 21: 252–268.
(21) RAINES, M. A.
1922. VEGETATIVE VIGOR OF THE HOST AS A FACTOR INFLUENCING SUSCEPTIBILITY AND RESISTANCE TO CERTAIN RUST DISEASES OF THE HIGHER PLANTS. I–II. Amer. Jour. Bot. 9: 183–203, 215–238, illus.
(22) REIMER, F. C.
1925. BLIGHT RESISTANCE IN PEARS AND CHARACTERISTICS OF PEAR SPECIES AND STOCKS. Oreg. Agr. Expt. Sta. Bull. 214, 99 pp., illus.
(23) ROSEN, H. R.
1929. THE LIFE HISTORY OF THE FIRE BLIGHT PATHOGEN, BACILLUS AMYLOVORUS, AS RELATED TO THE MEANS OF OVERWINTERING AND DISSEMINATION. Ark. Agr. Expt. Sta. Bull. 244, 96 pp., illus.
(24) ——— and GROVES, A. B.
1928. STUDIES ON FIRE BLIGHT: HOST RANGE. Jour. Agr. Research 37: 493–505, illus.
(25) STEWART, V. B.
1913. THE FIRE-BLIGHT DISEASE IN NURSERY STOCK. N.Y. (Cornell) Agr. Expt. Sta. Bull. 329, pp. [313]–372, illus.
(26) THOMAS, H. E., and THOMAS, H. E.
1931. PLANTS AFFECTED BY FIRE BLIGHT. Phytopathology 21: 425–435.
(27) TISDALE, W. H.
1917. FLAX WILT: A STUDY OF THE NATURE AND INHERITANCE OF WILT RESISTANCE. Jour. Agr. Research 11: 573–606.
(28) TULLIS, E. C.
1929. STUDIES ON THE OVERWINTERING AND MODES OF INFECTION OF THE FIRE-BLIGHT ORGANISM. Mich. Agr. Expt. Sta. Tech. Bull. 97, 32 pp., illus.
(29) WAITE, M. B.
1898. PEAR BLIGHT AND ITS TREATMENT. East. N.Y. Hort. Soc. Proc. 2: [779]–790, illus.
(30) WHETZEL, H. H.
1906. THE BLIGHT CANKER OF APPLE TREES. N.Y. (Cornell) Agr. Expt. Sta. Bull. 236, pp. [103]–138, illus.
(31) ——— and STEWART, V. B.
1909. FIRE BLIGHT OF PEARS, APPLES, QUINCES, ETC. N.Y. (Cornell) Agr. Expt. Sta. Bull. 272, pp. [31]–52, illus.
(32) WILEY, H. W.
1906–14. PRINCIPLES AND PRACTICES OF AGRICULTURAL ANALYSIS; A MANUAL FOR THE STUDY OF SOILS, FERTILIZERS, AND AGRICULTURAL PRODUCTS; FOR THE USE OF ANALYSTS, TEACHERS, AND STUDENTS OF AGRICULTURAL CHEMISTRY. 3 v., illus. Easton, Pa.

THE INTERRELATIONSHIPS OF BARK BEETLES AND BLUE-STAINING FUNGI IN FELLED NORWAY PINE TIMBER [1]

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INTRODUCTION

It is a well-known fact that trees injured by fire or heavy defoliation, or those felled by wind or in the course of lumbering operations, are very susceptible to attack by bark beetles, wood borers, and fungi. Such timber deteriorates rapidly and is often rendered almost worthless in one season. This fact is very important in forestry operations where it becomes necessary to leave cut logs in the woods for a season or more before they are taken to a mill.

The rapidity with which such timber may decline has been pointed out by Boyce (*1*),[3] who made a study of the deterioration of western yellow-pine trees felled in connection with control of the western pine beetle. He showed that the sapwood of such trees was completely blue-stained by the end of the first season after felling. Decay was also very rapid, especially in the sapwood, but was more pronounced the second season. However, Boyce did not mention any relationship between the bark beetles and the development of the stains and decay.

Graham (*6*), in a study of the felled tree trunk as an ecological unit, found that there was a definite succession of insects and fungi in the log as the chemical and physical character of the wood changed during the process of disintegration and decay. He pointed out that along with the insects typical of each region and stage of decomposition of the log there also were fungi equally typical of the parts where they occur. He further observed (*6*, *p. 399*) that "In some cases there is a distinct symbiotic relationship between wood inhabiting insects and fungi, as in the case of the ambrosia beetles; and in many other instances a looser type of symbiosis can be demonstrated." The details of these relationships, however, were not studied. The work reported in the present paper is the result of one phase of a project planned to study in some detail the interrelations of certain insects and fungi attacking felled logs kept under more or less controlled conditions.

PLAN OF EXPERIMENTS

The field experiments, initiated in the spring of 1931, were carried out at the Lake Itasca Forest Forestry Biology Station, Arago, Minn. Because of its long, smooth bole and relative freedom from

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[3] Reference is made by number (italic) to Literature Cited, p. 340.

limb scars, Norway pine, *Pinus resinosa* Ait., was chosen for the study. Healthy standing trees of suitable size were selected and felled on May 18 and 19. The trunks were immediately cut into logs 40 inches long and 7 to 12 inches in diameter. These logs, after treatment as indicated below, were placed in position under a slanting roof made of laths spaced so as to provide approximately 50 percent shade. The logs were placed upon wooden supports so that the north end was about 6 to 8 inches and the south end about 4 inches above the ground. The wooden supports had previously been painted with a copper

FIGURE 1.—A log from series C, sealed and enclosed in an 18-mesh screen-wire cage.

sulphate and linseed-oil paint as a precaution against spread of decay from the supports to the logs.

The logs were divided into 6 series of 8 logs each, except the check series, in which there were 16 logs. Each series was handled differently, as follows:

A. Not caged; no treatment (check series).
B. Not caged; ends and limb scars disinfected with a 2-percent aqueous solution of ethyl mercury chloride, then sealed with roofing pitch and covered with burlap.
C. Same as series B but enclosed in a cage of 18-mesh aluminum-coated screen wire (fig. 1).
D. No end treatment but enclosed in a cage of 18-mesh screen wire.
E. Not caged; ends and limb scars sprayed at frequent intervals throughout the summer seasons with a 2-percent aqueous solution of ethyl mercury chloride.
F. Same as series E but enclosed in a cage of 18-mesh screen wire.

It was planned that series A should serve as a check series, duplicating as nearly as possible the conditions in nature, being exposed to normal insect attack and any fungus spores disseminated by them as well as to infection by wind-blown spores through the exposed ends and limb scars.

Series B was designed to eliminate infection by wind-blown spores but to leave the logs exposed to normal insect attack. Thus, any fungus infection would be due to inoculum introduced by insects or entering through holes made by the insects.

In series C elimination of both fungus infection and insect attack was attempted.

Series D was planned to eliminate insect attack but to leave the logs exposed to infection by wind-blown spores.

Since logs sealed with pitch and burlap, as in series B and C, would obviously maintain a higher moisture content than unsealed logs and since this treatment might influence the results, the ends of the logs in series E and F were sprayed with a solution of ethyl mercury chloride but not sealed. This treatment, as was determined later, effectively prevented infection through the exposed ends and did not interfere with the normal drying of the logs.

One-fourth of the logs of each series were to be removed for examination at the end of each summer for 4 successive years. In this way the progress of insect attack and fungus infection under the different conditions could be followed and any associations between insect and fungus could be detected.

A record was kept of the particular tree from which each log was cut and the logs were so distributed in each series that any differences in results due to differences in individual trees would be evident. Each log was weighed at frequent intervals throughout the summer in order to determine the variations in weight.

In addition to the experiment outlined above, other experiments were started from time to time in which freshly cut logs were treated and caged, as in series C and F, and into which individual species of insects were introduced. In this way logs sealed against infection by wind-blown spores were subjected to attacks by individual species of insects. Thus the association between a given insect and fungus could be studied without being complicated by the presence of other insects or fungi.

Throughout the course of the investigation the progressive development of the insects and fungi was observed in logs that were barked and cut up at suitable intervals. Cultures of fungi were made from the logs and from the insects in various stages of development. Also insects in various stages of development were killed and embedded in paraffin for histological examination in order to determine the more intimate relationships between the insects and associated fungi. In this way it was hoped to gain a fairly accurate picture of the association of the insects and the fungi involved in the deterioration of the logs.

Two species of bark beetles, *Ips pini* Say and *I. grandicollis* Eich, were among the first insects to attack the exposed logs. This paper presents the results of a study of these two beetles and the fungi associated with them in their development in the logs.

LITERATURE RELATING TO BARK BEETLES AND BLUE STAIN

Apparently the bluing of timber was first described in 1878 by Hartig (*8*), who recognized its fungal nature and also mentioned the presence of insects in trees affected with blue stain. Münch (*12*), MacCallum (*11*), Wilson (*20*), and others in later years observed the association of blue stain with insect injury, but, apparently, made no study of the nature of the association. Von Schrenk (*19*), in 1903,

investigated more closely the relation of insects to the spread of blue stain. In addition to describing in detail the development of blue stain in and about the tunnels of *Dendroctonus ponderosae* Hopk., in trees of the western yellow pine, *Pinus ponderosa* Lawson, Von Schrenk dissected a number of beetles and attempted to isolate the blue-stain fungus from the intestinal tract and from feces. He failed to obtain the blue-stain fungus but found a characteristic bacterium present. He considered these trials "by no means conclusive for they were not exhaustive" and more work on a larger scale was projected, but the results were never published. The blue-stain fungus described by Von Schrenk was *Ceratostomella pilifera* (Fr.) Wint., and Rumbold (*17, p. 848*) is of the opinion that "possibly *C. pilifera* was a secondary blue stain in the wood that he used for starting his cultures and that the fungus that first stained it had lost its vitality by the time the wood reached the laboratory." This error and his failure to isolate the fungus from the beetles probably are responsible for Von Schrenk's conclusion (*19, p. 18*) that

> The spores of the "blue" fungus are probably blown about by the wind in countless thousands, and at the time of the beetle attack in July and August some of the spores lodge in the holes made in the bark of the living pine tree by the bark and wood-boring beetles.

After Von Schrenk's work in 1903 very little attention was given to this insect and fungus association until 1928, when Craighead (*5*) called attention to the constant association between tree-killing bark beetles (*Dendroctonus*) and blue stain. Craighead pointed out that the girdling of the trees by the bark beetles was not sufficient to cause the rapid death of the trees and suggested a symbiotic relation between bark beetle and blue-stain fungus in which the later contributed to the death of the tree. This was soon followed by the experiments of Nelson and Beal (*15*) and the more extensive studies of Nelson,[4] which showed that the girdling of pines by the tunnels of the bark beetles was insufficient to account for the rapid death of the tree. He showed by experimental inoculations that the blue-stain fungi would kill pines in a relatively short time without the help of the beetles, death being caused by the stoppage of sap flow through the stained wood. Nelson was able to isolate the blue-stain fungus from the bodies of beetles, and he concluded that it was commonly introduced under the bark by them.

Using material furnished by Craighead, as well as other material collected from various parts of the United States, Rumbold (*17*) found that *Ceratostomella pini* Münch was constantly associated with the attacks of *Dendroctonus frontalis* Zimm. and *D. brevicomis* Lec., and that a new species, *C. ips* Rumbold, was associated with *Ips grandicollis* and *I. calligraphus* Germ.

None of the above-named authors studied in detail the nature of the symbiosis between the beetles and the blue-stain fungi. However, a recent paper by Grosmann (*7*), who was working in Germany, deals in considerable detail with the nature of the symbiosis between bark beetles and fungi. She pointed out that the bark beetles carry yeasts as well as the blue-stain fungi, a fact also demonstrated by

[4] NELSON, R. M. EXPERIMENTS WITH BLUESTAIN FUNGI IN SOUTHERN PINES. Thesis, Ph.D., Univ. Minn. 1930. (In press.)

Person (*16*). Grosmann concluded that the insects are in no way dependent upon either the blue-stain fungi or the yeasts. They were considered as commensals.

RESULTS OF THE EXPERIMENTS

EXPERIMENTAL EVIDENCE THAT BLUE-STAIN FUNGI ARE INTRODUCED INTO THE LOGS BY BARK BEETLES

In the study of the relation of an insect to the spread and development of a disease, it is the authors' opinion that, in order to prove conclusively that an insect is an agent of dissemination and inoculation, the disease should be produced experimentally by visitation of the insect under controlled conditions with adequate checks. Although frequent or constant association of the insect with the disease may leave little doubt as to the role played by the insect, the proof is not complete until the relationship has been experimentally demonstrated. Insofar as the writers know this has not been done for the bark beetles and blue stain. One of the objects of these experiments was to determine to what extent bark beetles are dissemination and inoculation agents of blue stain.

The exposed logs that were not caged were found to be infested by bark beetles the latter part of May, only a few days after they were cut and put into position. The development of these and other beetles and the associated fungi was studied by frequent examination of extra logs exposed for that purpose.

On September 18, 1931, approximately 4 months after the experiment was started, the first set of 16 logs was removed and examined. The bark was removed from each log and the degree of insect infestation and the amount of blue stain and other fungus infection were noted. In doing this a 5-inch section of each end of the 40-inch log was disregarded, all notes being made on the central 30 inches. Since there was some variation in the size of the logs, the surface area of this region was recorded for each log. The degree and nature of insect and fungus infestation of each log are given in table 1. Additional evidence confirming the data given in table 1 was obtained from similar logs opened in the two following years and from logs opened while studying the progressive development of beetles and fungi, but since the evidence of association is so clear cut the detailed notes are given for one series only.

It will be seen from table 1 that blue stain always followed the attack of the 2 species of *Ips* and was absent from the logs that were protected from the insects by wire cages. Also, in those logs that became infested with only a few bark beetles that managed to get into the cages, the stain was limited to the immediate vicinity of the *Ips* tunnels, while the remainder of the log was free from stain. One log from each of the six different treatments was photographed, and these are shown in figure 2. Of particular interest is the log representing series D, in which is shown one nuptial chamber of *I. grandicollis* with one short side channel. Observe that blue stain has developed around this tunnel but is absent elsewhere. In figure 3 are shown cuts into logs from series E and F showing the development of blue stain in logs infested with *Ips* and the absence of stain in logs free of insect infestation.

TABLE 1.—*Degree and nature of insect infestation and fungus infection of the logs 4 months after the beginning of the experiment*

Log no.	Treatment and other data	Insect infestation	Fungus infection	Remarks
1	Not caged; no end treatment; surface area 735 square inches; tree no. 5.	*Ips pini*, 11 broods; *I. grandicollis*, 7 broods; *Monochamus* sp., 11 tunnels; *Rhagium* sp., 3 larvae; buprestid larvae, 2; a few small unidentified tunnels.	Blue stain abundant, but overgrown by dense mycelial mats of *Peniophora gigantea*; *Ceratostomella* perithecia scarce; yeast and a few unidentified fungi present; some green mold (*Trichoderma koningi*) in older tunnels; bark very loose.	*Peniophora* apparently entered through exposed ends, and by rapid invasion of sapwood inhibited the normal development of blue stain.
2	Not caged; no end treatment; surface area 787.5 square inches; tree no. 7.	*I. pini*, 7 broods; *I. grandicollis*, 12 broods; *Monochamus* sp., 8 tunnels; buprestid larvae, 2.	Essentially the same as no. 1	Essentially the same as no. 1.
3	Not caged; no end treatment; surface area 712.5 square inches; tree no. 5.	*I. pini*, 18 broods; *I. grandicollis*, 8 broods; *Monochamus* sp., 12 tunnels; buprestid larvae, 3; *Rhagium* sp., 2 larvae.	do	Do.
4	Not caged; no end treatment; surface area 682.5 square inches; tree no. 3.	*I. pini*, 18 broods; *I. grandicollis*, 7 broods; *Monochamus* sp., 17 tunnels; buprestid larvae, 5; *Rhagium* sp., 3 larvae.	do	Do.
7	Not caged; ends and limb scars sealed with pitch and burlap; surface area 817.5 square inches; tree no. 2.	*I. pini*, 43 broods; *I. grandicollis*, 17 broods; *Monochamus* sp., 15 tunnels; *Rhagium* sp., 3 larvae; buprestid larvae, 30.	Blue stain and yeast abundant and closely associated with *Ips* tunnels; other fungus infection insignificant or absent; bark loose.	Moisture content of the log much greater than that of logs with ends not sealed.
8	Not caged; ends and limb scars sealed with pitch and burlap; surface area 697.5 square inches; tree no. 6.	*I. pini*, 51 broods; *I. grandicollis*, 4 broods; *Monochamus* sp., 17 tunnels; buprestid larvae, 11.	do	Do.
9	Caged; ends and limb scars sealed with pitch and burlap, surface area 712.5 square inches; tree no. 6.	None	No blue stain present; 2 small spots of inner bark stained with a fungus forming small black sclerotia but no spores; apparently entering through minute cracks in the bark; bark not loose.	Do.
10	Caged; ends and limb scars sealed with pitch and burlap; surface area 705 square inches; tree no. 2.	do	No blue stain or other fungus infection; 100 percent clean; bark not loose.	Do.
11	Caged; no end treatment; surface area 735 square inches; tree no. 2.	*I. grandicollis*, 3 broods	Blue stain present only about tunnels of *I. grandicollis*. Some infection with a white basidiomycete (*P. gigantea*) entering through exposed ends; bark not loose.	One end of the cage was closed with muslin, a method which proved not to be always proof against smaller beetles; slower development of *Peniophora* as compared to logs 1 4 probably due to absence of *Ips*.
12	Caged; no end treatment; surface area 742.5 square inches; tree no. 7.	*I. grandicollis*, 3 broods, which developed only to egg-channel stage; *Pissodes* sp., 17 pupal chambers.	Blue stain and yeast associated with *Ips* egg channels but absent elsewhere; small amount of infection by *Peniophora* near exposed ends; bark not loose.	Do.

13	Not caged; ends and limb scars sprayed at intervals with ethyl mercury chloride; surface area 840 square inches; tree no. 5.	*I. pini*, 31 broods; *I. grandicollis*, 10 broods; *Monochamus* sp., 10 tunnels; buprestid larvae, 11; *Rhagium* sp., 4 larvae.	Blue stain and yeast abundant; spreading and fruiting to within 1 inch of ends, where it was checked by spray applied to end of log; some *Trichoderma* and other secondary fungi present; stain associated with *Monochamus* and buprestid tunnels only when these made contact with *Ips* tunnels; bark loose.	Spray applied at intervals to end of logs very effective against fungus infection, at the same time allowing the log to dry as rapidly as logs with no end treatment.
14	Not caged; ends and limb scars sprayed at intervals with ethyl mercury chloride; surface area 795 square inches; tree no. 7.	*I. pini*, 29 broods; *I. grandicollis*, 11 broods; *Monochamus* sp., 11 larvae; buprestid larvae, 5; *Rhagium* sp., 1 larvae.	....do....	Do.
15	Caged; ends and limb scars sprayed at intervals with ethyl mercury chloride; surface area 832.5 square inches; tree no. 7.	None....	One small spot of stain associated with crack in bark; fungus not identified, but not *Ceratostomella*; bark not loose.	Do.
16	Caged; ends and limb scars sprayed at intervals with ethyl mercury chloride; surface area 825 square inches; tree no. 5.	*I. grandicollis*, 4 broods, all on upper side of log.	Blue stain and yeast associated with *Ips* tunnels but absent elsewhere on log; bark not loose.	These beetles apparently entered through the end of cage covered with muslin.

As will be discussed in more detail later, cultures were made of the fungi found in these and certain other logs. It is sufficient to say here that blue-staining fungi were always found developing and spreading from the bark-beetle tunnels and that the evidence justifies the conclusion that they were introduced into the logs by the beetles and that they were rarely or never introduced in any other way. In addition to the blue-staining fungi, a characteristic yeast was found constantly associated with the beetle attacks. Several different kinds of bacteria, also, were isolated from freshly made tunnels, but these were not consistently present. In the older tunnels several

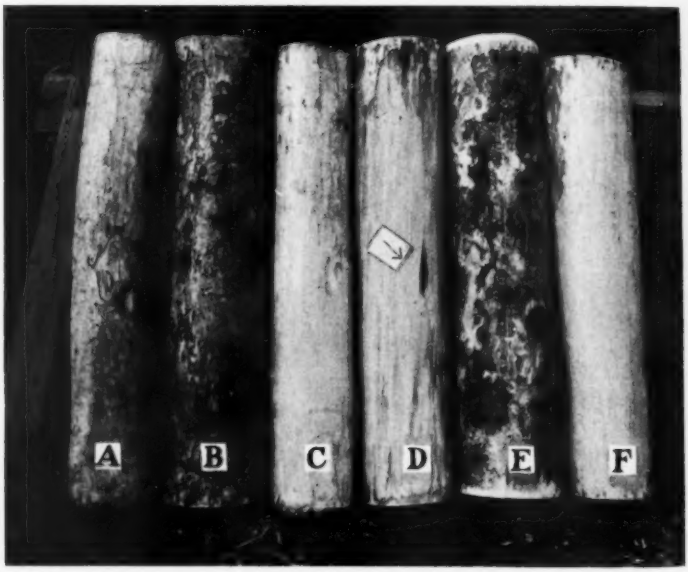

FIGURE 2.—One log from each of the six series; the arrow on the log from series D points to an area of blue stain surrounding an incomplete tunnel made by a single *Ips* beetle that managed to get through the cage.

other fungi were often present, the most common of which was a species of *Trichoderma*. It is very probable that these were also introduced by beetles or other insects, but they develop more slowly than the blue stain and are secondary in nature, apparently not being able to penetrate extensively into the woody tissue. The most common fungus entering through the exposed nontreated ends of the logs was *Peniophora gigantea* (Fr.) Massee. This fungus was not present in any of the logs with sealed or treated ends, but developed abundantly under the loosened bark of the logs with exposed ends and caused a rapid decay of the sapwood. Fruiting bodies were often found on the underside of the infected logs. Sporophores of this and other fungi sometimes grew out through holes made in the bark by insects, but there was no evidence that they were consistently introduced by bark beetles.

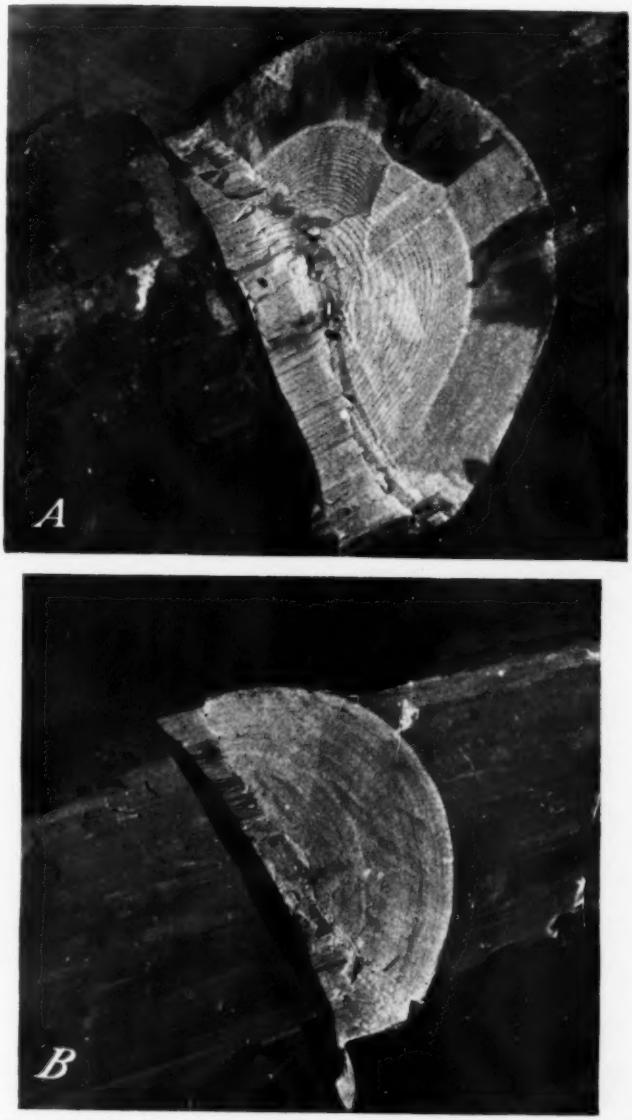

FIGURE 3.—Two logs cut to show presence or absence of stain in the sapwood: *A*, A log from series E, not caged; *B*, a log from series F, caged.

As a further check on the association of blue-stain infection and bark-beetle infestation, a log of each series was sawed in cross section as shown in figure 4. With the aid of a planimeter the area of blue-

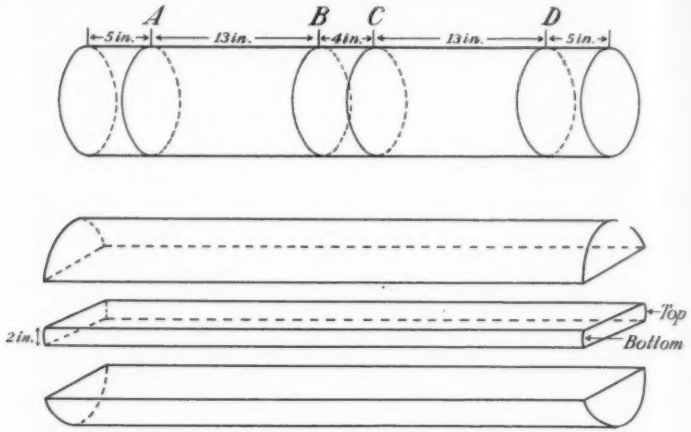

FIGURE 4.—Diagram to show how logs were cut for the measurement of the amount of stained sapwood.

stained wood, showing in the cross sections *A*, *B*, *C*, and *D*, was measured and calculated in terms of percentage of total area of sapwood in cross section. A plank 2 inches thick was sawed from each of the other logs as shown in figure 4. In the same manner the area

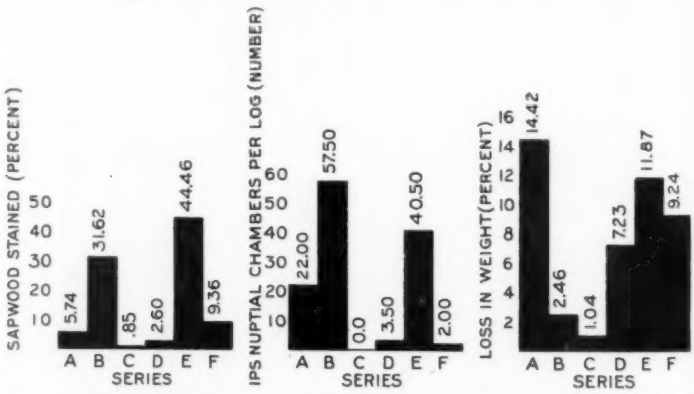

FIGURE 5.—Amount of blue stain in the logs of the different series, and the relation of insect infestation and moisture content of the wood to the prevalence of blue stain.

of stained wood, as revealed in longitudinal section, was calculated. The results of these measurements and also the degree of beetle infestation are shown graphically in figure 5. It will be seen that the amount of stain is closely correlated with the amount of bark-beetle infestation, except in the check logs 1, 2, 3, and 4. The extremely small amount of stain found in these logs that were heavily attacked by bark beetles was difficult to understand until it was noted that

the wood-rotting fungus *Peniophora gigantea* had penetrated practically all of the sapwood and, by its very rapid development, had inhibited the normal development of blue stain. This explanation of the absence of blue stain in these logs was verified the following season by observing the development of the fungi in similar logs at frequent intervals throughout the season.

Since it has been shown that the development of blue stain is influenced by the moisture content of the wood (*4*, *13*) and since the loss of water from the logs was influenced by the different treatments, the logs were weighed at frequent intervals until they were opened. Figure 5 shows graphically the percentage of loss in weight by the logs during the first summer of the experiment. This loss, for practical purposes, may be considered to be due almost entirely to loss of water. As a further check on the moisture content of the logs, six increment borings were made from a log of each series just before the bark was removed for examination. The approximate percentage of moisture in each sample (oven-dry basis) was as follows: A, 69; B, 110; C, 102; D, 70; E, 55; and F, 58. Although the nature of this determination is subject to considerable error, the relative values agree closely with the data obtained from the loss of weight. Since blue stain develops well at any moisture content between 55 and 110 percent, it is improbable that moisture content was a limiting factor in blue-stain development in these experiments. It is obvious from the data given in figure 5 that there is no correlation between the amount of blue stain and the moisture content of the logs of the different series.

Because of the several kinds of insects that entered the uncaged logs and the resulting overlapping of their tunnels, it was often difficult to interpret with assurance the significance of the fungi found associated with the tunnels. In the case of the bark beetles this difficulty was in part overcome by the fact that a few beetles entered the cages of logs 11, 12, and 16, which resulted in these logs being infested with a few isolated broods. In order further to overcome this difficulty additional logs were caged, with ends sealed or sprayed, and into each cage a few beetles of a single species were introduced. In all of these logs blue stain developed abundantly about all of the *Ips* tunnels and was absent on other parts of the log, verifying the conclusion that the blue-stain fungi are commonly introduced into the logs only by the bark beetles.

ASSOCIATION OF BARK BEETLES AND FUNGI

Parallel with the experiments reported above, the more intimate relationships between bark beetles and fungi were studied. Logs infested with bark beetles were opened at frequent intervals and the progress of fungus infection determined by microscopic and cultural studies. At the same time, insects in all stages of development were killed, fixed, and embedded in paraffin and were later studied by histological methods. In this manner, a fairly complete picture of the association between the bark beetles and the fungi was obtained. For the sake of brevity the results of both studies are presented together and the association is described in chronological sequence following the life cycle of the insects.

As stated in the foregoing, two species of bark beetles, *Ips pini* and *I. grandicollis* attacked the logs soon after they were cut and put in

place. Because of the similarity of development and fungus association of the two species, they will be discussed together, any observed differences being pointed out as the occasion demands.

The life history of these bark beetles is, briefly, as follows: Pupae and some of the young adults hibernate under the bark of logs or trees attacked during the previous summer. Young adult beetles often emerge from infested trees in the late fall months and enter the litter and duff under the trees and spend the winter there. They come out early the following spring and attack fresh trees or logs. The males find suitable logs or trees, and then each bores a hole through the bark (fig. 6, *A*, *a*) and excavates a broad, flat chamber in the inner bark and cambium region. This chamber is known as the "nuptial chamber" (fig. 6, *A*, *b*). Several females soon join the male in this chamber and each female constructs a long tunnel known as an "egg tunnel", extending out from it (fig. 6, *A*, *c*). The pattern in which these egg tunnels radiate from the nuptial chamber is characteristic of the species. As the female extends her egg tunnel she makes small niches or pockets along each side of it (fig. 6, *A*, *d*). An egg is placed in each of these niches and covered with boring dust or frass. The eggs hatch in a few days and the young larvae begin burrowing tunnels (fig. 6, *A*, *e*) in the inner bark at approximately right angles to the egg tunnels. These larval tunnels increase in size as the larvae grow. The larvae become full grown and ready to pupate in about 3 weeks. Each larva then excavates a small oval chamber at the end of its tunnel. This is the pupal chamber (fig. 6, *B*). The larva then transforms to the pupal stage and later to the adult, the length of this transformation period varying with the season of the year. The young adults do not immediately emerge from the log but burrow about under the loosened bark, feeding extensively. After this period of feeding, they bore exit holes through the bark and fly away in search of a new tree or log. There are 2 generations a year in Minnesota, 1 emerging in midsummer, the other in fall and early spring.

The life histories and habits of *Ips pini* and *I. grandicollis* are rather similar. One of the chief differences is in the arrangement of their tunnel patterns. In the case of *I. pini* there are usually 3 to 6 egg tunnels, each made by a different female, radiating in a star-shape pattern from the nuptial chamber. *I. grandicollis* forms usually only 2 or 3 egg tunnels from each nuptial chamber, and these tunnels extend in a longitudinal direction very nearly parallel with the grain of the wood. This difference in tunnel pattern is important because the extent of the circumference of a log involved by a brood of *I. pini* is naturally considerably greater than that invaded by *I. grandicollis*. Since the blue-stain fungi grow most slowly in a tangential direction, the more spreading tunnels of *I. pini* usually result in a greater cross-sectional area of blue-stain infection and consequently a more rapid death of infested trees.

In May and early June 1931 many beetles were caught as they alighted upon the logs cut from a freshly felled Norway pine. Some of these were killed and fixed for histological study. Cultures were made from some, and others were dissected and examined under the microscope. Microscopic examination of these beetles frequently disclosed fungus spores adhering to the legs, wings, and other external parts. Several different kinds of spores were found, but the most abundant and most characteristic were at once recognized as ascospores

of the *Ceratostomella* found in the logs infested by the two species of *Ips*. This fungus has since been described and named by Rumbold (17) as *Ceratostomella ips*. Cultures made from the beetles, both internally and externally, yielded this fungus along with certain

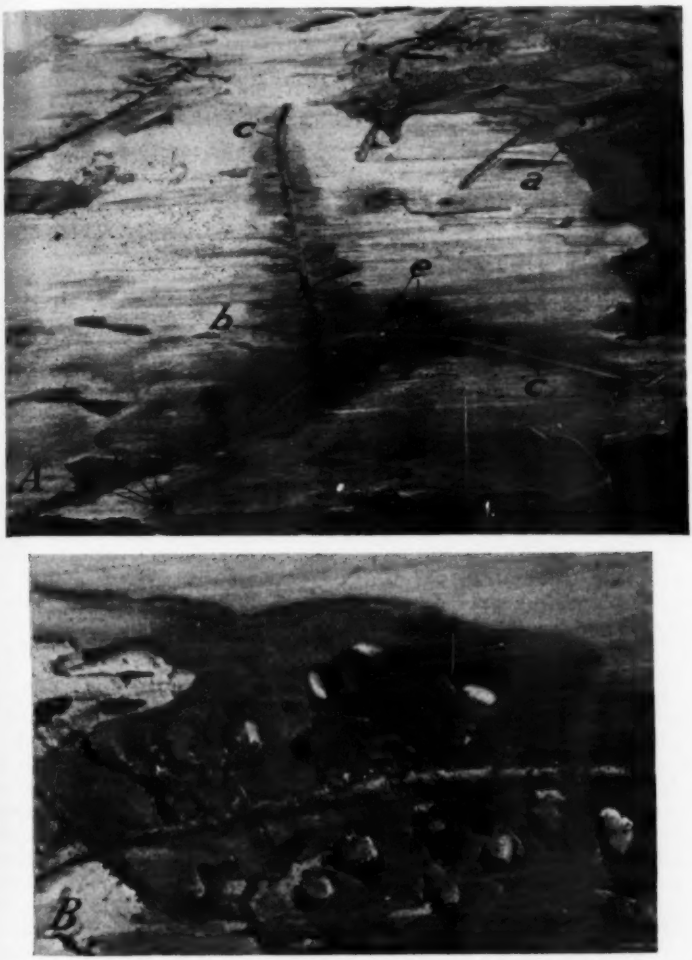

FIGURE 6.—*A*, Portion of a Norway pine log with bark removed to show tunnels of *Ips pini*: *a*, An entrance hole; *b*, nuptial chamber; *c*, 1 of 3 egg channels; *d*, egg niches; *e* larval tunnels; *B*, pupal chambers and pupae of *I. grandicollis*.

characteristic yeasts. Bacteria and other fungi were frequently obtained, but *C. ips* and the yeasts were universally present. The most frequently occurring fungus other than these two was *Trichoderma koningi* Oudem. found, also, in nearly all of the old bark-beetle tunnels. The universal occurrence of these fungi in and on the bodies

of the beetles caught as they were alighting on the freshly cut logs supports the evidence that the fungi are introduced into the logs by the insects.

Among the fungi isolated from the beetles and from the stained wood adjacent to the beetles were several cultures closely resembling *Ceratostomella ips*, but bearing only conidia. The conidia were often formed on typically *Graphium*-like coremia, but on malt agar the sporophores were more commonly shortened to form tuberculate clusters. These were cultured on various media and under various conditions, but they could not be induced to form perithecia. These cultures could be identified only as species of *Graphium*. On account of the similarity of these cultures to *C. ips*, it was thought that they might be the haploid form of this fungus. (Buisman (*3*) has recently shown that when several strains of *G. ulmi* are mixed in culture, perithecia of *Ceratostomella* are formed.) But when several of these cultures were mixed, no perithecia were formed.

As a further check on the identity of these imperfect forms a single perithecium of *Ceratostomella ips* was placed in a drop of sterile distilled water until the ascospores oozed out of the ostiole. With the aid of a Chambers micromanipulator 15 individual ascospores were picked up and cultured. Nine of these single ascospore cultures produced perithecia in abundance and also formed a few conidia. Six of the cultures formed conidia and were similar in all respects to the *Graphium* cultures isolated previously. When each of these six cultures was grown in combination with each of the remaining imperfect cultures only conidia were formed. Moreover, when an imperfect culture was grown on agar side by side with a perfect form each retained its characteristic type of fructification. If the imperfect forms were haploid and the perfect forms diploid, there was no evidence of diploidization of the haploid form. It would appear from this evidence that the *Graphium* cultures isolated from stained wood and from the beetles were specifically identical with *C. ips* but lacked the ability to form perithecia.

Histological study of the beetles also revealed the universal presence of spores of the blue-stain fungi, both externally and internally. The beetles were killed and fixed in Dietrich's solution in a partial vacuum. They were embedded in paraffin, and sections 12 to 15 microns thick were cut and stained with the Gram-Weigert stain. This procedure revealed the ascospores of *Ceratostomella ips* adhering in clumps to various parts of the exoskeleton and also scattered throughout the intestinal tract (fig. 7). In many specimens fragments of perithecia were found, showing that the beetles feed upon these fruiting bodies. Staining reactions gave no indication that the spores were digested or otherwise injured by passage through the body of the insect. To test this point further a number of beetles were caught in sterile glass vials and held until pellets of feces were obtained. Some of these were plated on 1-percent malt-extract agar, and cultures of *C. ips* were obtained from a high percentage of them. Examination of the pellets under the microscope also disclosed the characteristic ascospores of this fungus. When the pellets were crushed and placed in hanging drops of sterile water, the spores germinated and grew normally, thus proving conclusively that they were not injured by passage through the intestinal tract of the beetles.

Cultures made from the bark and sapwood adjacent to freshly made nuptial chambers yielded *Ceratostomella ips* and yeast in nearly every

case, showing that the spores germinate soon after they are introduced. In all such chambers pellets of feces are found adhering to the walls of the chamber. The moist bark evidently furnishes sufficient moisture for germination of the spores and subsequent growth of the fungi. Although no proof can be presented, it is quite probable that spores adhering to the exoskeleton of the beetles are brushed off on the moist wood, where they would germinate and start infection.

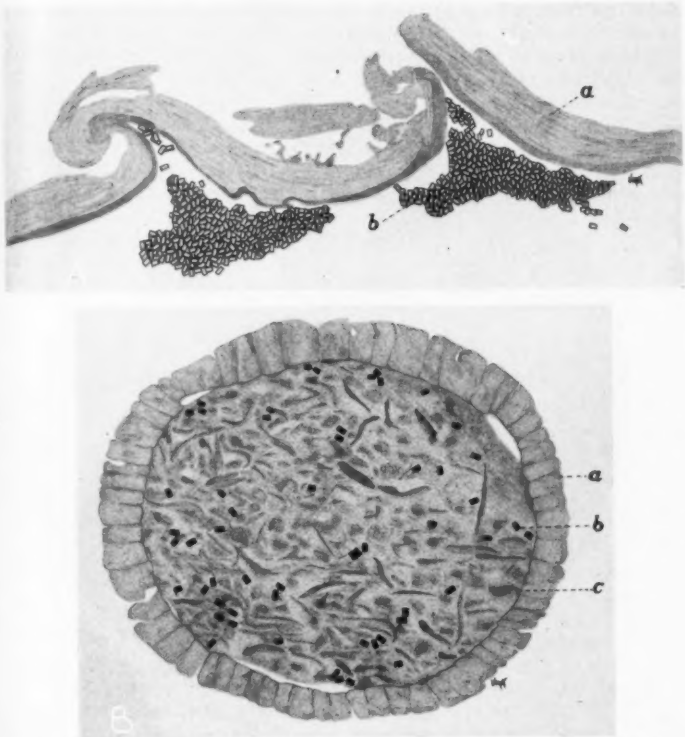

FIGURE 7.—Detailed drawings showing the spores in and on the body of mature beetles: *A*, Masses of ascospores adhering to the abdomen of a beetle, exoskeleton (*a*), masses of ascospores (*b*); approximately × 400; *B*, cross section of the midintestine, showing (*a*) wall of intestine, (*b*) ascospores of *Ceratostomella ips*, (*c*) partly digested wood fragments; approximately × 400.

Another interesting means of distributing the inoculum within the tunnel was discovered while beetles caught on the surface of the logs were being dissected. It was observed that from one to a dozen or more small mites were attached to the ventral part of the thorax or in the concave wing declivities of nearly all the beetles examined. These obviously had attached themselves to the beetles before they emerged from the old infested logs. Careful examination showed mites running about in nearly all freshly made tunnels. They were also found in great abundance in old tunnels. Apparently, they leave the beetles when they enter the log and feed on the fungi and decom-

posing bark tissue. When the beetles of the new brood are ready to emerge, some of the mites attach themselves to the beetles and are thus carried to new trees or logs. Microscopic examination of the mites taken from the beetles, as well as cultures made from them, showed that spores of the blue-staining fungus were often disseminated by them.

Of the various fungi introduced into the log by the bark beetles, the yeasts apparently develop most rapidly at first. When the outer bark is removed from a freshly made tunnel, such as the one shown in fig-

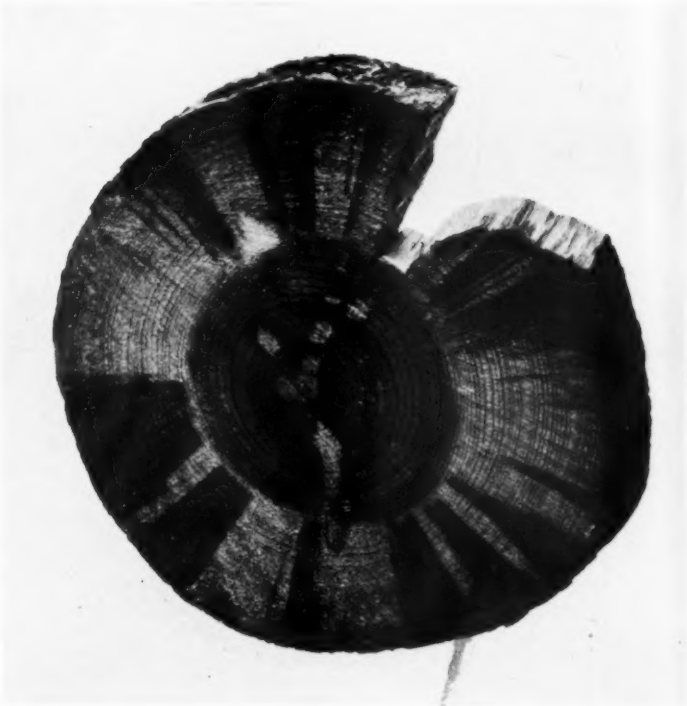

FIGURE 8.—Section cut from a log of Norway pine to show the development of blue stain in the sapwood

ure 6, *A*, it is seen that the inner bark is stained brown for some distance around the tunnel. Isolations from the advancing border of this stained bark frequently yield pure cultures of yeast, while the blue-staining fungi can be found only in the bark and wood closer to the tunnel. As time goes on, the blue-stain fungi spread rapidly in the sapwood and inner bark. The rate of spread is most rapid in a radial plane, up and down the log as well as toward the center. In advanced stages stained areas appear as wedges in the sapwood when seen in cross section (fig. 8). The blue stain, however, does not affect the heartwood and its radial development ceases sharply when the heartwood is reached.

As stated above, the eggs of the bark beetles are deposited singly in small niches in the sides of the egg channel. Each egg is covered with a soft plug of sawdust. Cultures made from this sawdust plug show that it is always contaminated with yeast and other fungi. When the eggs are aseptically removed and placed on agar, it is evident they also are frequently surface-contaminated with yeast, *Ceratostomella*, and other fungi. The eggs are very delicate and easily injured, but a limited number of cultures made from surface-sterilized eggs show that they are internally sterile. As the young larvae develop and bore out a tunnel in the inner bark, the tissue surrounding the tunnel is stained brown. Yeasts can nearly always be found in the stained tissue, but such tissue is often sterile, indicating that the brown stain may be due in part to oxidation. When larvae were killed and embedded in paraffin, stained sections often revealed yeast cells in their intestinal tracts. In many cases, however, no yeast was found in the bodies of the larvae.

Shortly after the larvae pupate the blue-stain fungi begin to sporulate. The nature and amount of sporulation is more or less dependent upon the immediate environment. By this time the bark usually is becoming loose and there is a space of varying width between it and the wood. If the log is exposed to direct sunlight, its upper surface may dry out and there may be little surface growth or fructification of the fungi.

Ceratostomella ips forms two kinds of spores. Conidia are formed usually in the typical *Graphium* manner and coremia are often found in the old egg channels, the larval tunnels, or in the pupal chambers (fig. 9, *A*). The spores are often formed in the pupal chambers while they are still occupied by the pupae, so that the newly formed beetles are surrounded by a mass of sticky conidia. *C. ips*, however, in nature produces perithecia more commonly than conidia. The perithecia are formed frequently on the sides of the old egg channels, with their long black beaks pointing toward the center of the channels (figs. 9, *B*, and 10). They may be formed also in any crack in the inner bark or even completely embedded in the soft, partially decayed tissue of the inner bark. When moisture conditions are right, the ascospores ooze from the beaks of the perithecia in white sticky masses (figs. 9, *B*, and 10).

When transformation is complete the newly formed beetles begin feeding. They wander through the old egg channels (fig. 9, *B*) and also make new feeding tunnels in the inner bark. In doing this they take many spores into their digestive tract and many more stick to the external parts of the body, so that, when the beetles emerge from the log, they are thoroughly infested with fungus spores.

Although *Ceratostomella ips* was the blue-staining fungus most frequently associated with the bark beetles, another apparently undescribed fungus frequently was found that caused a stain indistinguishable from that caused by *C. ips*. When this fungus was present it usually predominated, and in many tunnels examined *C. ips* apparently was entirely absent. The fungus was first observed fruiting in the pupal chambers of *Ips pini* and was later found associated with several broods of *I. grandicollis*. Only the conidial fructification has been observed, and this is formed most frequently in the pupal chambers and in the adjacent larval tunnels. Sporula-

tion begins shortly after pupation and, by the time the mature beetle is formed, the walls of the pupal chamber are lined with a white waxy mass of conidia (fig. 11). Observations have shown that these conidia often form part of the first food consumed by the newly

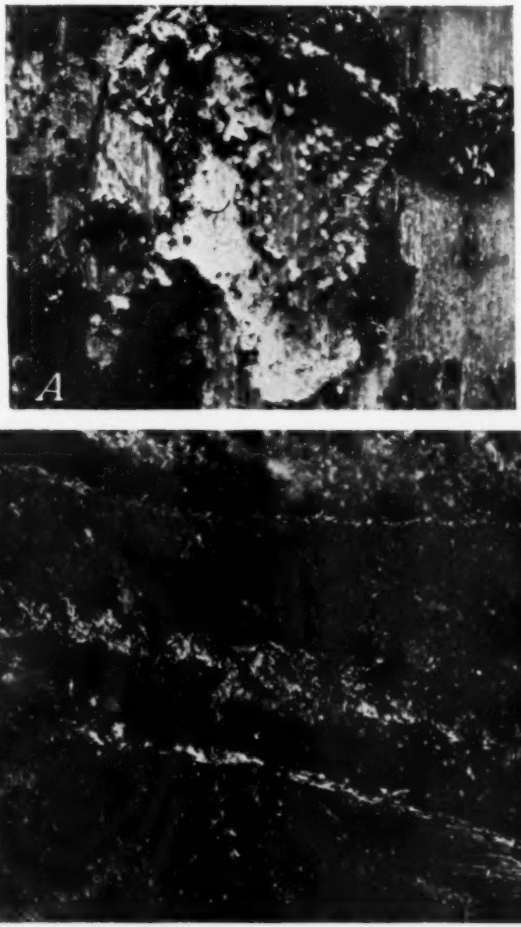

FIGURE 9.—*A*, *Graphium*-like coremia formed by *Ceratostomella ips* in a pupal chamber of *Ips pini*, approximately × 10. *B*, Bark beetle (*I. grandicollis*) in an old egg channel; note the perithecia of *C. ips* lining the tunnel, with their beaks bearing glistening masses of spores all pointing toward the center of the channel. Such beetles become contaminated with the spores both internally and externally.

formed beetle. The rather large hyaline globose to pyriform conidia (fig. 12, *A*) are formed on the ends of short, erect palisaded sporophores, forming a compact cushion (fig. 12, *B*). In its general aspects this fungus resembles the so-called "ambrosia" fungi described by Hubbard (*10*), Neger (*14*), Schneider-Orelli (*18*), and

others and associated with the ambrosia beetles. Cultures of the fungus were obtained by picking up single conidia and also by making

FIGURE 10.—Perithecia of *Ceratostomella ips* in a beetle tunnel; note the glistening mass of sticky ascospores on the tips of the perithecia.

tissue cultures from blue-stained wood adjacent to the beetle tunnels. At least two different strains of the fungus were isolated that differed somewhat in spore size but were identical in other respects. The

FIGURE 11.—Two pupal chambers of *Ips pini* showing immature beetles surrounded by masses of spores of *Tuberculariella ips*.

spores of one strain were somewhat smaller and more globose than those of the other. There was considerable variation in size and shape of spores in any given culture. Some were distinctly globose,

while others were long and pyriform. Spores formed on agar cultures also were distinctly more globose than those formed in nature. The spores ranged from 7.9μ to 22.5μ in width and from 13.3μ to

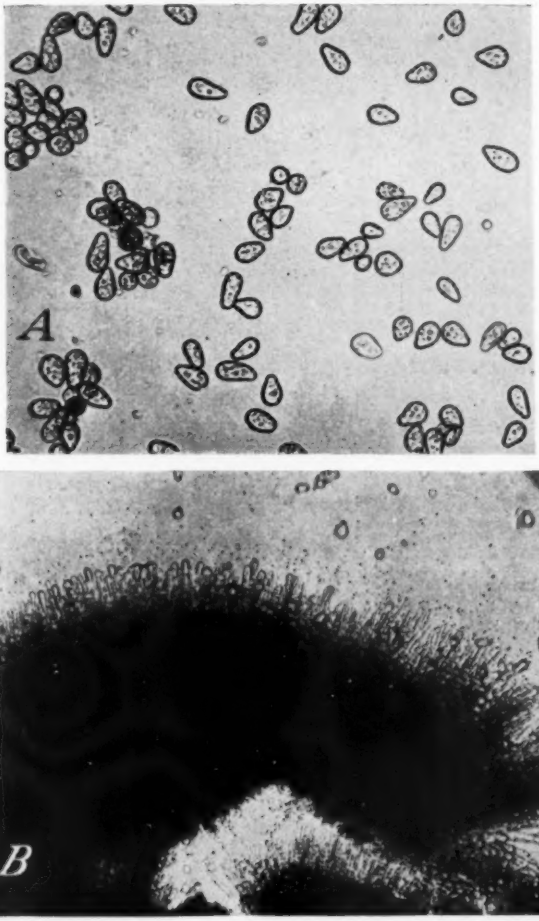

FIGURE 12.—*A*, Conidia of *Tuberculariella ips;* approximately × 130. *B*, Sporodochium of *T. ips* showing the palisade arrangement and terminal position of spores; approximately × 50.

23.8μ in length. The sporodochia were white and waxy, but there was no distinct mucus holding the spores together. The fungus appears to be a species of the genus *Tuberculariella* Von Höhnel (*9*). Because of its close association with the two species of *Ips*, the name *Tuberculariella ips* is proposed. A brief technical description is given below.

TECHNICAL DESCRIPTION

Tuberculariella ips, n.sp.

Conidia hyaline, globose to pyriform, usually apiculate at the narrow end, ranging from 7.9μ to 22.5μ in width and from 13.3μ to 23.8μ in length, borne singly and successively at the ends of unbranched septate conidiophores; sporodochia white and waxy but not mucilaginous. Young colonies on agar colorless, later changing to black; scant gray aerial mycelium; young hyphae hyaline, old hyphae brown; mycelium 2.7μ to 6.7μ in diameter. In agar cultures conidia borne terminally on simple branches of the mycelium; sporodochia sometimes formed in old cultures.

On sapwood of *Pinus* spp. infested with *Ips pini* Say or *I. grandicollis* Eichh. at Itasca Park, Arago, Minn. Causes distinct blue stain of sapwood, and fruits abundantly in pupal chambers and larval tunnels of the insects.

LATIN DIAGNOSIS

Sporodochia alba cerea haud glutinosa; conidia hyalina, globosa vel pyriformia, plerumque apiculata ad extremitatem angustiorem, 7.9μ—22.5μ lata, 13.3μ—23.8μ longa, ad apices conidiophororum simplicium septatorum singulatim iterum atque iterum lata.

Coloniae in "agar" cultae primum hyalinae deinde nigres centes, mycelio aerio tenui griseo hyphis primum hyalinis deinde brunneis; mycelio 2.7μ—6.7μ crasso, conidiis ad apices ramorum simplicium mycelii latis; sporodochiis interdum in culturis antiquis factis.

As stated above, the newly formed beetles frequently find themselves completely surrounded by masses of the conidia of this fungus. Numerous observations have shown that the beetles often feed upon these spores, frequently completely cleaning out the pupal chambers before leaving them. Dissections and histological studies also have shown large numbers of these spores in the intestinal tracts of the beetles (fig. 13). Although some of the spores appear to be destroyed by the passage, many of them evidently are not injured. A thick gelatinous cell wall usually is present on such spores stained in the intestinal tract, and the spores show no signs of injury. Microscopic examination of freshly emerged beetles shows spores adhering to the external parts of the beetles, but, unlike the ascospores of *Ceratostomella ips*, they are readily washed off when the beetles are passed through the solutions in preparation for embedding in paraffin.

By artificial inoculation it was proved that this fungus would cause typical blue stain indistinguishable from that caused by *Ceratostomella ips*. The fungus was grown also on many different kinds of media under different conditions, including irradiation with ultraviolet light, in an unsuccessful effort to induce the production of sexual spore forms. It appears, then, that the fungus is another true blue-staining fungus actively spread by these two species of bark beetles. The same fungus has been found also in association with *Ips pini* in white-pine windfalls. The factor or factors influencing the prevalence of the two blue-staining fungi and determining which one will predominate are not known.

As previously mentioned, cultural experiments showed that yeasts are universally associated with the bark beetles. They are the first to multiply extensively in the bark adjacent to freshly made tunnels. Yeasts also have been isolated from the sapwood, but here they are soon outgrown by the blue-staining fungi. The yeasts are present in great quantity in the inner bark in later stages of the beetles' development and are taken into the bodies of the mature beetles during their feeding period just prior to emergence from the log. The histological studies show yeast cells in varying amounts always

present in the food contents of the intestinal tracts of freshly emerged beetles.

On several occasions small masses of oblong hyaline conidia were found in the old tunnels of *Ips pini*. Cultures made from single spores formed brown mycelial cultures on malt agar but did not produce blue stain on wood. Examination of the mycelium showed the presence of clamp connections, and older cultures formed what

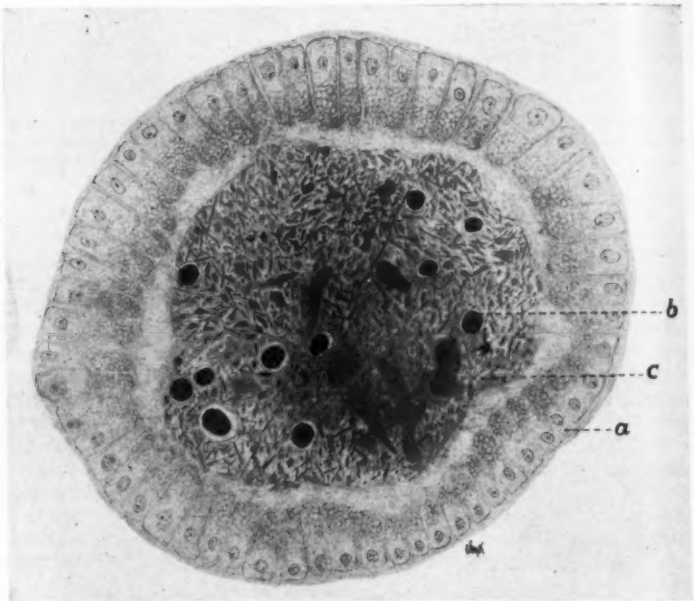

FIGURE 13.—Highly magnified section of the intestinal tract of a mature beetle (*Ips pini*) showing the presence of viable spores of *Tuberculariella ips*: *a*, Intestinal wall; *b*, conidium of *T. ips*; *c*, partly digested wood fragments. Approximately × 75.

appeared to be abortive poroid fruiting bodies. The identity of the fungus and its significance are not known.

In isolating fungi numerous bacterial colonies were found in the plates. These were so variable and inconsistent in their occurrence that they were considered of little significance.

In addition to the bark beetles and the mites previously mentioned, a number of insects of various types frequently were found inhabiting old tunnels. They were mainly predators or parasites of the bark beetles. Since these insects enter the logs only after the bark beetles and associated fungi have become thoroughly established, it is thought that they are of secondary importance only.

DISCUSSION

The facts presented above show quite clearly that blue-staining fungi are definitely associated with *Ips pini* and *I. grandicollis* and that they are disseminated and introduced into trees or felled logs by

these beetles. The nature of the fructification of the blue-staining fungi makes it appear highly improbable that wind is of any significance in disseminating them. The results of the controlled experiments with caged and uncaged logs, some with sealed ends and some with ends exposed, bear out this conclusion. Although the blue-staining fungi are the most obvious ones associated with the beetles, cultural studies show that other fungi are present with more or less regularity, even when other insects are absent. When one looks at the inner bark of a log at the time beetles are emerging and sees the many different kinds of fungi growing and sporulating, it is obvious that several different fungi must be disseminated with some degree of regularity. It is possible that some of these organisms are of greater significance than would be indicated by general observations. On the other hand, evidence, both cultural and histological, tends to show that the blue-staining fungi and the associated yeast fungi are predominant, both in the amount of inoculum carried by the bark beetles and in the rapidity of their development in the log.

As would be expected, other fungi develop as the disintegration of the inner bark advances and as the bark is loosened from the wood. These, without doubt, play a part in the ultimate destruction of the log and cannot be ignored completely. The most common of these "secondary" fungi is the green moldlike fungus identified as *Trichoderma Koningi*. It fruits abundantly in the old tunnels before and after the beetles have emerged. The spores are borne loose and dry in a manner favoring wind distribution, but since it has been isolated from the bodies of freshly emerged beetles, probably it also is frequently disseminated by them.

The constant association of fungi with these bark beetles naturally raises the question of the symbiotic relationships between them. As previously mentioned, Grosmann (7) has already considered this question in her study, which dealt primarily with *Ips. typographus* L. and to a lesser extent with several other species of bark beetles. The blue-staining fungus associated with *I. typographus* was described by Grosmann and named *Leptographium penicillatum*. In addition, 2 or 3 characteristic yeasts were always present. Grosmann reached the conclusion that no true symbiosis existed between the beetles and their associated fungi. She concluded from her experiments that the beetles were not dependent upon the blue-stain fungi and that if any benefits were derived from the association they were all in favor of the fungus. She also concluded that the yeasts were in no way necessary for the development of the beetles and were to be considered as commensals.

Although the writers have not made extensive experiments dealing with the nutrition relations of the bark beetles and the associated fungi, observations and histological studies make it seem very unlikely that the beetles utilize the fungi extensively as food. In the examination of several hundred prepared slides of insects in all stages of development there was no indication that the fungi were digested. Yeast cells were often found in the intestinal tract of the larvae, but they showed no signs of disintegration and appeared to pass through uninjured. They were frequently entirely absent, and when present did not appear to be multiplying rapidly. No fungi of any sort were found inside the body of the pupae. In adult beetles both yeast cells and spores of the blue-staining fungi were generally

present, but they, likewise, appeared to be uninjured. Since neither of these fungi destroy wood to any appreciable extent and apparently do not multiply in the body of the insects, it is highly improbable that they digest the wood particles taken in by the insects. The feeding experiments of Grosmann (*7*) would tend to show that the action of the fungi on the tissues of the bark do not make it more suitable as food for the beetles. Since her experiments did not include the entire life cycle of the insects and were not carried out under fungus-free conditions, this conclusion cannot be accepted as beyond question.

One other factor, apparently overlooked by Grosmann, is the effect of the fungi on the immediate environment of the developing brood of beetles. Until it is possible to bring about the development of a brood of beetles in bark entirely free of fungi, it cannot be safely concluded that the fungi do not in some way influence favorably the development of the beetles. In the writers' experiments in which logs were protected from insects by cages and from fungus infection by sealing or spraying the ends, the bark did not separate from the wood as did that of infested logs. When the tissue of such bark as a medium for beetle development is compared with that overgrown by fungi and separating from the wood, it is readily seen that the two environments are strikingly different. Moreover, most conifers secrete resin into freshly made wounds, and in living trees such resin secretions are often able to overcome the attack of individual insects or broods. Many bark beetles are known to attack living trees most successfully when the trees are weakened by drought or other factors. It is very probable that reduced resin flow or moisture content of the inner bark may be the determining factor in such cases. Nelson [5] has shown that the blue-stain fungi, in the absence of beetles, interrupt the flow of sap in infected trees, causing a rapid decrease in moisture content and usually resulting in death within a few months. Obviously this reduces the flow of resin. Thus beetles aided by the blue-stain fungi are able to attack certain trees which without such aid they could not successfully attack. Such benefits derived from the fungi by the beetles would certainly come within the broader concept of symbiosis, as generally used. That the fungi derive benefit from the association through dissemination by the beetles and by introduction into the tissues of the inner bark is obvious from the facts mentioned above. Their special adaptation to dissemination in this way would indicate a symbiotic relationship of long standing.

Person (*16*) has demonstrated recently that the inner bark of western yellow pine when fermented by the yeast associated with *Dendroctonus brevicomis* Lec., is more attractive to this beetle than the nonfermented bark. This fact is offered as an explanation of the selection of certain trees for attack by the beetle. Person believes that, in trees weakened by drought or other abnormal conditions, a limited amount of respiratory fermentation results in the production of volatile aldehydes or esters that attract a few beetles in the immediate vicinity of the trees. These introduce the yeast, which increases the rate of fermentation and results in an increased amount of the attractive substance sufficient to attract beetles from a greater

[5] Nelson, R. M. See footnote 4.

distance. If this explanation is correct, the yeasts also must be considered as truly symbiotic with the beetles.

Buchner (*2*) and others have described several wood-inhabiting beetles that possess special anatomical modifications of the female definitely adapted to insure the perpetuation of symbiotic fungi by contamination of the egg either before or after oviposition. When this study was started it was suspected that some such structure would be found in the bark beetles. Careful study of many sections of the insects in all stages of development has, however, revealed no such structures. This accords with the observations of Grosmann (*7*) on *Ips typographus*. Transmission of the fungi from one generation to another apparently is accomplished entirely as indicated previously, namely, by means of spores and yeast cells either adhering to the external parts of the insect or passing through the intestinal tract and being expelled in the feces in newly made tunnels. Male and female beetles appear equally effective in introducing the fungi. Both yeasts and blue-stain fungi have been isolated from freshly made nuptial chambers containing only the male insect. The fungi have been isolated also from the bodies of both male and female beetles. In several instances in caged logs large patches of blue stain developed from nuptial chambers into which no females entered.

SUMMARY

A study of two species of bark beetle (*Ips pini* Say and *I. grandicollis* Eichh.) and the fungi associated with them has been made as the first part of a general investigation of the interrelations of insects and fungi in the deterioration of felled logs of Norway pine.

Experimental evidence is presented showing that these bark beetles introduce blue-staining fungi into the logs and that the fungi are rarely, if ever, introduced in any other way.

Two different blue-staining fungi were found associated with these bark beetles. The most prevalent of the two is *Ceratostomella ips* Rumbold, the fungus isolated by Rumbold from the galleries of *Ips calligraphus* and *I. grandicollis*. The second apparently has not previously been reported. It is briefly described in this paper as *Tuberculariella ips*, n.sp.

Certain cultures of *Graphium* isolated from stained wood and from the beetles proved to be identical with *Ceratostomella ips*, although they could not be made to produce perithecia. Of 15 cultures derived from single ascospores of *C. ips*, 6 formed only conidia and were identical with the *Graphium* cultures previously isolated. The remaining 9 cultures formed both conidia and perithecia. Perithecia were not produced when the 6 *Graphium* cultures were mated in all combinations.

In addition to the blue-staining fungi, characteristic yeasts were constantly associated with the beetles.

The fungi are introduced by either male or female beetles, and they begin to grow in the inner bark and sapwood soon after introduction. The yeast fungi grow more rapidly at first in the inner bark, but the blue-stain fungi spread more extensively in the sapwood.

Mites are frequently introduced into the logs by the beetles. The mites attach themselves to the underside of the thorax and in the concave wing declivities, where they are not easily brushed off.

When the beetles enter a log some of the mites leave the beetles and move about in the tunnels as they are constructed. Yeast cells and spores of the blue-staining fungi were found adhering to the bodies of the mites. The mites probably aid in distributing the spores about the beetle tunnels.

The blue-staining fungi sporulate profusely during the pupation of the bark beetles. *Ceratostomella ips* forms perithecia more commonly than conidia, but typical *Graphium* coremia are often found in the old egg channels or in the pupal chambers. The perithecia of *C. ips* are usually formed on the walls of the old egg channels with their beaks pointing toward the center of the channels. When moisture conditions are favorable, the spores ooze from the tips in sticky masses. The newly formed beetle leaves its pupal chamber and feeds extensively under the bark before emerging. In doing this it brushes against the sticky spores which adhere to the body of the beetle. Examination of the contents of the intestinal tract of beetles shows that ascospores and even parts of the perithecia are eaten. Large quantities of spores and yeast cells are found in the intestinal tracts of the beetles. These bear no signs of injury, and germination experiments show that they are still viable after passage through the body of the beetles.

The second blue-stain fungus forms masses of sticky conidia in the pupal chambers and in the old egg channels during the pupation period. These spores also adhere to the bodies of the beetles and are passed through the intestinal tract uninjured.

Yeast cells frequently are found in the intestinal tract of the larvae, where they also are apparently not injured. No fungi were found inside the body of the pupae.

Histological study of the mature beetles revealed no anatomical modification to insure transmission of the fungi to the young.

The eggs of the beetles were internally sterile, although yeast and fungus mycelium were abundant in the sawdust plugs covering the eggs in the niches.

Although no nutritional symbiosis could be demonstrated between the beetles and their associated fungi, the relationship is considered as one of true symbiosis in the broader sense. The fungi obviously derive benefit in being disseminated by the beetles and in being introduced into the inner bark of the logs or susceptible trees. The blue-staining fungi, by inhibiting the flow of sap, in all probablility make living trees more favorable for beetle development, and by aiding in the decomposition of the inner bark cause it to separate from the wood, creating a more favorable environment for the development of the insect broods. Until a brood of beetles can be reared in a fungus-free log, it cannot safely be concluded that the fungi are not necessary for the normal development of the beetles.

LITERATURE CITED

(1) Boyce, J. S.
1923. The deterioration of felled western yellow pine on insect-control projects. U.S. Dept. Agr. Bull. 1140, 8 pp., illus.

(2) Buchner, P.
1930. Tier und pflanze in symbiose. Aufl. 2, völlig umgearb. und erweiterte von Tier und Pflanze in Intrazellularer Symbiose. 900 pp., illus. Berlin.

(3) Buisman, C.
1932. ceratostomella ulmi, de geschlachtelijke vorm van graphium ulmi schwarz. Tijdschr. Plantenziekten 38: 1–5, illus.
(4) Colley, R. H., and Rumbold, C. T.
1930. relation between moisture content of the wood and blue stain in loblolly pine. Jour. Agr. Research 41: 389–399, illus.
(5) Craighead, F. C.
1928. interrelation of tree-killing bark beetles (dendroctonus) and blue stain. Jour. Forestry 26: 886–887.
(6) Graham, S. A.
1925. the felled tree trunk as an ecological unit. Ecology 6: 397–411, illus.
(7) Grosman, H.
1930. beiträge zur kenntnis der lebensgemeinschaft zwischen borkenkäfern und pilzen. Ztschr. Parasitenk. 3: [56]–102, illus.
(8) Hartig, R.
1878. die zersetzungserscheinungen des holzes der nadelholzbäume und der eiche in förstlicher, botanischer und chemischer richtung. 151 pp., illus. Berlin.
(9) Höhnel, F. von
1915. beiträge zur mykologie. ix. über die gattung myxosporium link. Ztschr. Gärungsphysiol. 5: [191]–215.
(10) Hubbard, H. G.
1897. the ambrosia beetles of the united states. U.S.Dept.Agr., Div. Ent. Bull. (n.s.) 7: 9–30, illus.
(11) MacCallum, B. D.
1922. some wood-staining fungi. Brit. Mycol. Soc. Trans. 7: 231–236, illus.
(12) Münch, E.
1907–8. die blaufäule des nadelholzes. Naturw. Ztschr. Forst u. Landw. 5: 531–573, illus., 1907; 6: 32–47, 297–323, illus., 1908.
(13) ———
1909. untersuchungen über immunität und krankheitsempfänglichkeit der holzpflanzen. Naturw. Ztschr. Forst u. Landw. 7: 54–75, 87–114, [129]–160, illus.
(14) Neger, F. W.
1911. zur überträgung des ambrosiapilzes von xyleborus dispar. Naturw. Ztschr. Forst u. Landw. 9: 223–225, illus.
(15) Nelson, R. M., and Beal, J. A.
1929. experiments with bluestain fungi in southern pines. Phytopathology 19: 1101–1106.
(16) Person, H. L.
1931. theory in explanation of the selection of certain trees by the western pine beetle. Jour. Forestry 29: 696–699.
(17) Rumbold, C. T.
1931. two blue-staining fungi associated with bark-beetle infestation of pines. Jour. Agr. Research 43: 847–873, illus.
(18) Schneider-Orelli, O.
1911. die überträgung und keimumg des ambrosiapilzes von xyleborus (amsandrus) dispar f. Naturw. Ztschr. Forst u. Landw. 9: 186–192, illus.
(19) Von Schrenk, H.
1903. the "bluing" and the "red rot" of the western yellow pine, with special reference to the black hills forest reserve. U.S.Dept.Agr., Bur. Plant Indus. Bull. 36, 40 pp., illus.
(20) Wilson, M.
1922. the bluing of coniferous timber. Roy. Arbor. Soc. Trans. 36: 82–92.

SELENIUM INJURY TO WHEAT PLANTS AND ITS INHIBITION BY SULPHUR[1]

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INTRODUCTION

Selenium is absorbed by plants from soils containing it (*3, 5, 17, 18, 19, 22, 23*),[3] and relatively small amounts are injurious (*1, 6, 7, 11, 18, 19, 20, 23*). It is a natural constituent of the soil of certain areas (*17*) where the vegetation is toxic to animals, causing a livestock disease (*12, 16*) now suspected to be due to ingested selenium.[4][5][6] It has also been found in plants growing along drainage canals from seleniferous mineral springs (*22*). It occurs in soils to which it has been added inadvertently as an impurity in superphosphate and ammonium sulphate (*18, 19*) and in those contaminated with fungicides and insecticides containing it (*4, 14, 15, 21*).

Cameron (*3*) first suspected that the injury to plants caused by selenium might be due to its replacement of sulphur in some organic compound. Brenner (*2*) was interested in the possibility that it might replace sulphur in the metabolism of sulphur bacteria. Later, Levine (*11*) referred to the possibility that selenium might replace sulphur in plant metabolism. The recent finding that selenium absorbed by wheat occurs in intimate association with the protein is suggestive in this connection (*15*). The present paper reports experiments showing a relation between the concentration of available sulphur in the substratum and the degree of injury produced in wheat plants by sodium selenate.

METHODS

Hard Federation wheat, *Triticum vulgare* Vill., was grown in the greenhouse in Keyport clay-loam soil, quartz sand, and culture solutions, to which sodium selenate ($Na_2SeO_4.10H_2O$) was added as the source of selenium. The selenate was dissolved in sufficient water to produce a desirable soil moisture and thoroughly mixed into the soil to insure uniform distribution. In the quartz-sand experiments, the selenate was added to the nutrient solution and poured on the sand in the pot.

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[2] The writer is indebted to J. E. McMurtrey, Jr., of the Bureau of Plant Industry, K. F. Kellerman, formerly of the Bureau of Plant Industry, and W. O. Robinson, of the Bureau of Chemistry and Soils, for valuable suggestions contributing to the progress of the experimental work, and to S. C. Salmon for assistance in the preparation of the manuscript.

[3] Reference is made by number (italic) to Literature Cited, p. 356.

[4] In cooperative investigations, based on observations and suggestions by K. W. Franke and others of the South Dakota Agricultural Experiment Station, K. F. Kellerman, formerly of the Bureau of Plant Industry, and H. G. Knight, of the Bureau of Chemistry and Soils, the cause of this disease, which occurs in certain restricted areas of the northern Great Plains, has apparently been traced to selenium occurring naturally in the soil and absorbed by the vegetation on which the animals graze or are fed.

[5] FRANKE, K. W., RICE, T. D., JOHNSON, A. G., and SCHOENING, H. W. REPORT ON A PRELIMINARY FIELD SURVEY OF THE SO-CALLED "ALKALI DISEASE" OF LIVESTOCK. U.S. Dept. Agr. Cir. 320, 8 pp., illus. 1934.

[6] WILLIAMS, K. T., and BYERS, H. G. OCCURRENCE OF SELENIUM IN PYRITES. Indus. and Engin. Chem., Analyt. Ed. 6: 296–297. 1934.

Applications of elemental sulphur and gypsum were thoroughly mixed into the soil before the selenate solution was added. The soluble sulphates were added, in the case of both soil and sand cultures, in the solution containing the selenate.

In the water cultures, seedlings were grown with nutrient solutions containing different concentrations of selenate and sulphate. They were germinated on blotters or paper towels, then transferred to wide-mouthed 600-cc Erlenmeyer flasks. There were three seedlings in a flask, each supported in a hole of the paraffined cork. They grew for the period of the experiment (4 to 6 weeks) without change of the nutrient solution except for weekly additions of iron citrate, and frequent volume adjustment with distilled water. The chemicals used were of c. p. grade, dissolved in distilled water. The nutrient solutions of a given experiment all contained equal amounts of potassium phosphate, calcium nitrate, iron citrate, and, in addition, such quantities of ammonium nitrate, magnesium nitrate, ammonium sulphate, and magnesium sulphate as to supply equal magnesium and nitrogen but different sulphur contents as desired. Two series of solutions, designated A and B, were used in the different experiments. Their composition is given in table 1.

TABLE 1.—*Composition of nutrient solutions*

Solution type	Cubic centimeters of stock solutions taken for dilution to 19 liters [1]					
	M/5 KH_2PO_4	M/2 $Ca(NO_3)_2.4H_2O$	M/1 NH_4NO_3	M/5 $Mg(NO_3)_2.6H_2O$	M/5 $(NH_4)_2SO_4$	M/5 $MgSO_4.7H_2O$
Series A:						
No sulphur	95	95	38	95	0	0
32 p.p.m. sulphur	95	95	57	0	0	95
96 p.p.m. sulphur	95	95	19	0	190	95
Series B:						
No sulphur	95	95	76	95	0	0
10 p.p.m. sulphur	95	95	82	65	0	30
32 p.p.m. sulphur	95	95	95	0	0	95
96 p.p.m. sulphur	95	95	57	0	190	95
192 p.p.m. sulphur	95	95	0	0	475	95

[1] All nutrient solutions contained 100 cc M/60 iron citrate also.

EFFECTS OF SELENIUM ON WHEAT PLANTS

The characteristic symptom of selenium injury produced in wheat by sodium selenate is a snow-white chlorosis of the leaves (fig. 1), evidently like that described by Turina (*23*) for barley seedlings injured by sodium selenate. With concentrations of selenium as high as 20 p.p.m. in the soil, part or all of the leaf may be white, maintaining its normal turgidity for some time before withering. Usually the tip remains green, as does the midvein. Sometimes the white leaves are notched as though broken by inhibition of cell division along the edge. Leaves less severely affected are streaked with white, the veins remaining green.

Highly toxic concentrations (above 30 p.p.m. selenium in the clay-loam soil) may cause rapid yellowing and death of seedlings without the snow-white chlorosis. With concentrations which are lower but sufficiently toxic to cause severe injury, chlorosis appears at the base of the first leaf, 2 or 3 days after emergence. With still lower con-

centrations chlorosis first appears at the base of the second leaf. The plants are stunted but sometimes survive. With concentrations as low as 15 p.p.m. the seedlings appear normal until chlorosis appears at the base of the third leaf, unaccompanied by stunting or

FIGURE 1.—Typical selenium chlorosis of young wheat leaves caused by the addition to the soil, 1 month before the photograph was taken, of 20 p.p.m. selenium (as sodium selenate). The plant represented at *A* was 3 months old when the selenate was added; that at *B*, 2 months; that at *C*, 1 month. In the case of that represented at *D*, the selenium was added when the seed was sown. The lower leaves of *A*, *B*, and *C* remained green, having been formed before the selenate was added. (The light color at the tip of the lower leaf of *A* is due to yellowing and is not the typical white selenium chlorosis.)

other visible injury. When sodium selenate is added to soil in which older plants are growing, the first chlorosis appears about 2 weeks later at the base of the new unfurling leaf. Those leaves which are fully formed at the time the selenate is added turn yellow if the con-

centration is sufficiently high, but they never show the typical white chlorosis.

A striking symptom occurring sporadically in the selenized seedlings—notably those in quartz sand, rarely those in soil—is a pink coloration at the base of the chlorotic leaves. The intensity of the color varies from a very faint tint to a rose pink suggestive of the color of precipitated selenium. More or less similar coloration of leaves and roots of plants injured by selenium, and of bacteria grown in media containing selenium compounds, has been observed by other investigators and has usually been interpreted as due to reduction of absorbed selenium compounds to red colloidal selenium, precipitated within the cell (*1*, *8*, *9*, *10*, *11*, *18*, *23*). The pink coloration of the leaves fades a few days after its appearance. Levine (*10*) attributes similar fading of the pink color in selenized bacterial cells to disappearance of the selenium as a result of alkylation to a volatile organic compound, possibly the ethyl selenide of mercaptanlike odor which is formed by the action of bacteria on media containing selenium compounds. That some such volatile compound is formed in selenized wheat plants is indicated by their characteristic odor, which becomes very strong when the cut plants have been in a closed container for a few hours.

Selenized plants that are not too severely damaged tend to outgrow the chlorotic condition. The white leaves eventually die, chlorotic areas never becoming green; but successive leaves are less injured and the plants may eventually appear entirely normal. Apparently this recovery is not due entirely to exhaustion of the selenium in the soil, for seedlings grown subsequently in the same pots may again be chlorotic. It seemed that it might be due to reduced susceptibility to injury as the plants became older. However, in experiments in which sodium selenate (at the rate of 20 p.p.m. selenium) was added to pots containing plants from 1 to 3 months old, the new leaves which appeared after about 2 weeks were white at the base, regardless of the age of the plants (fig. 1). It appears therefore that the recovery of chlorotic plants cannot be attributed to reduction in susceptibility with increasing age. This tendency to recover from a condition which has the appearance of irreparable injury was even more marked in sand than in soil cultures. Thus seedlings nearly as severely injured as those in figure 2, *B*, eventually formed only green leaves, developed normally, and produced as much grain (2.44 g per plant) as did the controls (2.36 g per plant).

RELATION OF SULPHATE TO SELENIUM TOXICITY IN SAND CULTURES

In the first experiment to give evidence of the inhibition of selenium toxicity by sulphur, the plants were grown in pots containing 7 kg of sand, to which 0.033 g sodium selenate had been added when the seeds were sown. Three nutrient solutions were used, containing 0, 32, and 96 p.p.m. sulphur, respectively, in the form of magnesium and ammonium sulphates (nutrient series A, table 1). One pot of each of the three groups was left as a control without selenium.

The condition of the plants a month after sowing is shown by representative pots in figure 2, the controls without selenium being in the lower row. The plants with no sulphate (*A*) were dead, those with 32 p.p.m. sulphur as sulphate (*B*) were extremely chlorotic and

stunted, while those with 96 p.p.m. sulphur (*C*) showed no chlorosis and were like the controls (*D*, *E*, *F*) in height and development. That the poor growth of *A* and *B* was due to selenium injury and not to sulphur deficiency was evident from the fact that, except for a

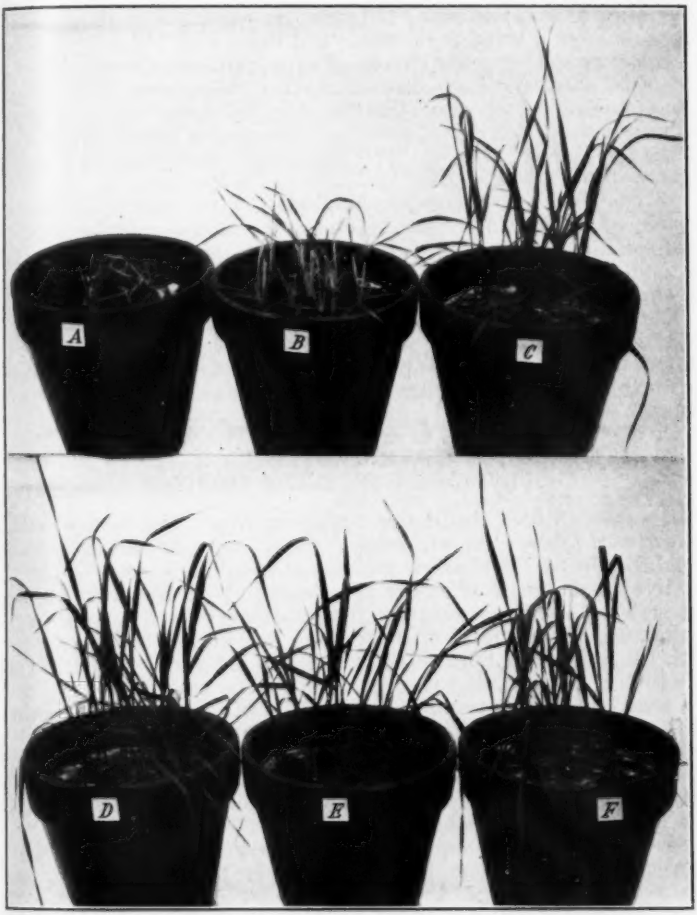


FIGURE 2.—Relation of the sulphate content of the nutrient solution to the injury produced by sodium selenate (0.033 g) on wheat grown in quartz sand (7,000 g). (Surface of sand covered with black sand for photographing.) Lower row, *D*, *E*, and *F*, are controls without selenate. *A*, *D*, No sulphur; *B*, *E*, 32 p.p.m. sulphur as magnesium sulphate; *C*, *F*, 96 p.p.m. sulphur as magnesium and ammonium sulphates.

lighter green color, the unselenized control plants without sulphate (*D*) remained almost normal throughout the growth period.[7]

Traces of chlorosis later appeared on a few of the plants of the high-sulphate group (*C*); so the experiment was repeated, with the inclusion

[7] Their growth suggested an extraneous source of sulphur, either from fumigants used in the greenhouse or from impurities in the chemicals.

of a still higher sulphur concentration (192 p.p.m.). The amount of selenium in each pot (0.033 g sodium selenate) remained the same (nutrient series B, table 1). After 5 weeks the plants in the pots containing selenium with no sulphur were dead; those with 10 p.p.m. sulphur were very chlorotic and severely stunted; those with 32 p.p.m. were stunted and had many chlorotic leaves; a few of those with 96 p.p.m. showed a trace of chlorosis; and those with 192 p.p.m. showed no chlorosis and remained like the controls without selenium throughout their development. As before, the no-sulphur control grew almost normally,[8] showing that sulphur deficiency was not specifically responsible for the chlorosis and stunting of the selenized plants of the low-sulphur groups. This experiment was repeated 6 months later, with identical results.

An application of ammonium sulphate (1 g per pot) to some of the injured plants after they had become very chlorotic largely inhibited chlorosis on subsequent leaves and so brought about marked improvement in the appearance of the plants as compared with those given an equivalent amount of ammonium nitrate.

The hydrogen-ion concentrations of the nutrient solutions (pH 5.0 $\pm$0.1 initially) were in no case correlated with the differences in the growth of the plants. The pH values of the sand extracts (obtained by washing the sand with a minimum of distilled water) were all between 6.1 and 7.0.

RELATION OF THE SELENIUM-SULPHUR RATIO IN WATER CULTURES TO SELENIUM TOXICITY

In water cultures also it was apparent from the growth of plants grown with various concentrations of sulphate that toxicity was determined not by the absolute amount of selenium present but by the relative amount with reference to sulphur. With no sulphur (nutrient series A, table 1) even the lowest concentration of selenium, 1 p.p.m., was quickly fatal; with 32 p.p.m. sulphur, there was no injury with 1 p.p.m. of selenium and only slight injury with 3 p.p.m.; whereas with 96 p.p.m. sulphur the plants showed no trace of chlorosis until the relatively high selenium concentration of 10 p.p.m. was reached. Minimum perceptible injury thus appeared where the ratio of selenium to sulphur was approximately 1 to 10. Differences in the acidity of the solutions were not responsible for these differences in toxicity, the pH values all being approximately 5.0 initially, and between 5.2 and 5.8 after the plants had grown 3 weeks.[9]

Another experiment was undertaken with a wider range of sulphate concentrations, the range of selenium concentrations set up in each (nutrient series B, table 1) being based on the assumption that the selenium-sulphur ratio at the point of minimum toxicity was about 1:10. The results of this experiment are shown in table 2. The point of minimum detectable injury to the plants (slight stunting with an occasional trace of chlorosis) was at 0.1 p.p.m. selenium with no sulphur present, at 1 p.p.m. with 10 p.p.m. sulphur, at 3 p.p.m. with 32, at 11 p.p.m. with 96, and at 18 p.p.m. with 192 p.p.m. sulphur,

[8] See footnote 7.

[9] In a number of preceding experiments with a different nutrient solution, chlorosis had always appeared with 8 p.p.m. selenium and with equal consistency had failed to appear with 4 p.p.m. On calculating the sulphur concentration in the solution, it was found to be 64 p.p.m., giving a selenium-sulphur ratio of 1:8 where chlorosis appeared and a ratio of 1:16 where there was no injury. The results thus fell into line with the generalization established by later experiments.

the corresponding ratios for the four series with sulphur being roughly 1:10, 1:11, 1:9, and 1:11, respectively. The severity of chlorosis and stunting increased in each series as the selenium-sulphur ratio increased, early death occurring where the ratio was 1:2. There was no injury in any series where there was 12 or more times as much sulphur as selenium.

TABLE 2.—*Relation between sulphur concentration in the nutrient solution and the toxicity of selenium to wheat seedlings, as estimated from chlorosis and stunting of the tops* [1]

[May 11 to June 8, 1933]

Selenium (in p.p.m.) as selenate	Selenium injury to wheat seedlings grown with the following concentrations (in p.p.m.) of sulphate sulphur—				
	0	10	32	96	192
0	0	0	0	0	0
.001	0				
.005	0				
.01	0				
.05	0				
.1	+				
.2		0			
.4		0			
.5	Dead.				
.6		0			
.8		0			
1.0	Dead.	+	0		
2.0		++	0		
3.0		+++	+		
4.0		++++	++		
5.0		Dead.	++		
6.0			+++	0	
7.0			+++	0	
8.0			++++	0	
9.0			++++	0	
10				0	
11				+	
12				++	
13				++	0
14				+++	0
16					0
18					+
20					++
22					++
24					+++
26					+++
28					++++

[1] 0=no injury; +=minimum detectable injury, (viz, slight stunting with not more than an occasional trace of chlorosis); ++=moderate chlorosis and stunting; +++=severe chlorosis and stunting; ++++=extreme chlorosis and stunting.

The experiment was repeated 6 months later, the range of selenium concentrations in the high-sulphate solutions being extended. Similar nutrient solutions were used, all with pH values near 5.1 (nutrient series B, table 1). A duplicate series containing 32 p.p.m. of sulphur was set up, made alkaline (pH 8.1) by the addition of sodium hydroxide. After 2 weeks markedly better root development in this series led to the addition of enough sodium hydroxide to the others to decrease their acidity to pH 7.0, since optimum growth conditions were desired. Neither these differences in the reaction of the solutions nor the differences in growth conditions obtaining in the greenhouse changed the selenium-sulphur relationship established by the preceding experiments. The data are presented in table 3.

The selenium concentrations producing minimum detectable injury ranged from 0.1 p.p.m. in the absence of sulphur to 18 p.p.m. with 192 p.p.m. sulphur. In all four sulphur-containing solutions the lowest

concentration of selenium producing visible injury was approximately one-tenth that of the sulphur present. As the proportion of selenium to sulphur increased, injury appeared earlier and with progressive severity, until at ratios greater than about 1 to 3 growth was inhibited. The appearance of the plants of each series at the age of 5 weeks is shown in plates 1 and 2.

TABLE 3.—*Relation between sulphur concentration in the nutrient solution and the toxicity of selenium to wheat seedlings, as estimated from injury to roots and tops* [1]

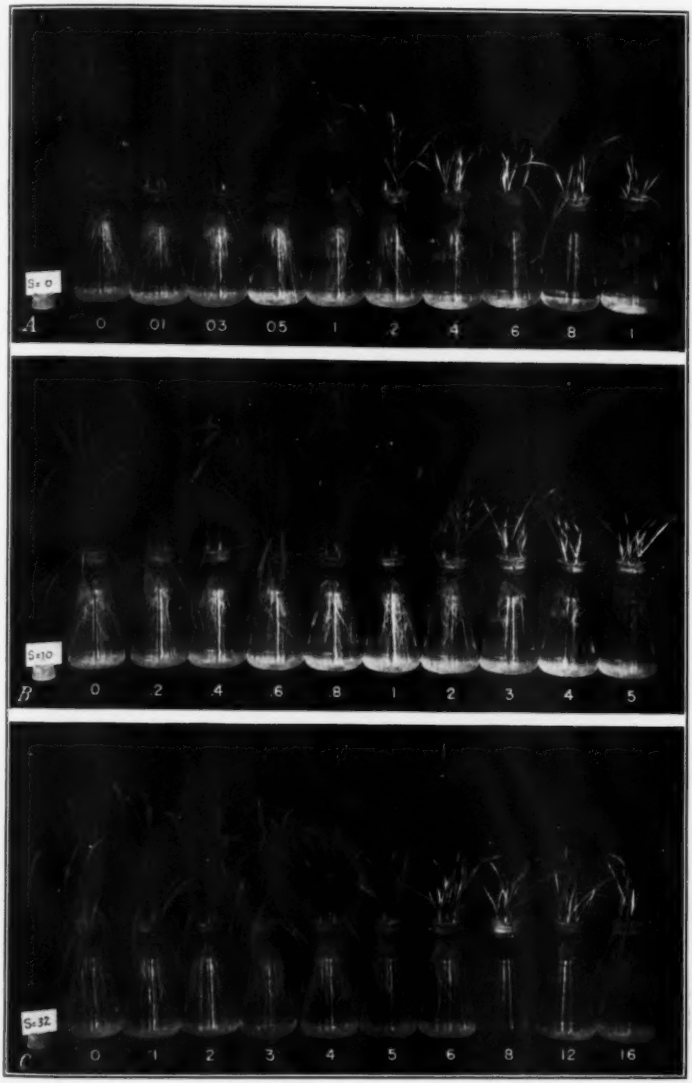
[Oct. 29 to Dec. 11, 1933]

Selenium (in p.p.m.) as selenate	Selenium injury to wheat seedlings grown with the following concentrations (in p.p.m.) of sulphate sulphur—				
	0	10	32	96	192
0	0	0	0	0	0
.01	0				
.03	0				
.05	0				
.1	+				
.2	+++	0			
.4	++++	0			
.6	+++++	0			
.8	+++++	0			
1.0	Dead.	+	0		
2		+++	0		
3		++++	+		
4		Dead.	++		
5		Dead.	+++		
6			++++		
8			++++	0	
9				0	
10				+	
11				++	
12			+++++	++	0
14				+++	0
16			Dead.		0
18					+
20				++++	++
24					+++
30				+++++	++++
48				Dead.	+++++
96					Dead.

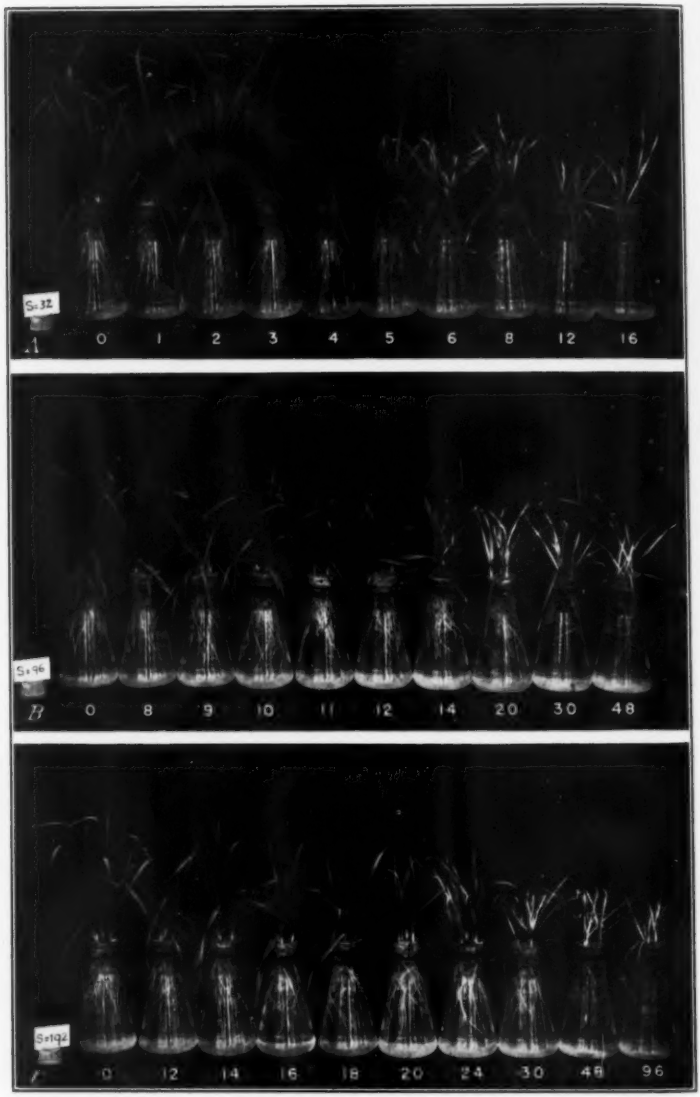
[1] 0=no injury; +=minimum detectable injury (viz, slight stunting of tops with only an occasional trace of chlorosis); ++=slight chlorosis and stunting, no obvious root injury; +++=moderate chlorosis and stunting, slight root injury; ++++=severe chlorosis and stunting, root injury pronounced; +++++=severe chlorosis and stunting, development of both roots and tops inhibited. (The "dead" plants still had a trace of green on the youngest leaves.)

When the plants were discarded a month later, after having grown considerably larger, the relative degrees of injury in the different flasks were still approximately the same. There was almost complete inhibition of both top and root growth wherever the ratio was as high as 1:2; stunting and chlorosis between 1:3 and 1:8; minimum detectable injury between 1:9 and 1:11 in the different series; and no injury in any case with a ratio of 1:12 or less. There was some variation in the condition of the plants having the same selenium-sulphur ratio in the different series, notably greater injury in the high-sulphate series than at the corresponding ratios in the low-sulphate series; but the generalizations established earlier still obtained.

Large differences in acidity developed in the flasks of the different series. The solutions in each flask of the high-sulphur series, 96 and 192 p.p.m., were very acid (below pH 5), except those in which little growth occurred, where the acidity decreased progressively in proportion to the degree of injury to the plants. At the highest selenium-



Toxicity of sodium selenate to wheat in nutrient solutions containing: *A*, No sulphur; minimum detectable injury at 0.1 p.p.m. selenium. *B*, 10 p.p.m. sulphur as magnesium sulphate. Minimum detectable injury at 1 p.p.m. selenium. *C*, 32 p.p.m. sulphur as magnesium sulphate. Minimum detectable injury at 3 p.p.m. selenium. (Figures under flasks indicate p.p.m. selenium.)



Toxicity of sodium selenate to wheat in nutrient solutions containing: *A*, 32 p.p.m. sulphur as magnesium sulphate, made alkaline (pH 8.1) by the addition of sodium hydroxide. Minimum detectable injury at 3 p.p.m. selenium. *B*, 96 p.p.m. sulphur as magnesium and ammonium sulphates. Minimum detectable injury at 10 p.p.m. selenium. *C*, 192 p.p.m. sulphur as magnesium and ammonium sulphates. Minimum detectable injury at 18 p.p.m. selenium. (Figures under flasks indicate p.p.m. selenium.)

sulphur ratio, where almost no growth occurred, the pH value was unchanged (7.0). In the other series containing less sulphur, the pH values were all between 6 and 7.

Vigor of growth in the high-sulphur (192 p.p.m.) control cultures without selenium was only slightly reduced by the excess sulphate and high acidity; and the only evidence of sulphur deficiency shown by the corresponding controls without any sulphur was a somewhat paler green color, stiffer, more erect leaves, and slight stunting.

FIGURE 3.—Inhibition of injury produced by sodium selenate (20 p.p.m. of selenium), added to the soil (6,000 g) when the plants were 3 months old, by the simultaneous addition of ammonium sulphate (3 g) (table 4): *A*, Ammonium sulphate alone—normal yield of grain; *B*, sodium selenate alone—heads sterile; *C*, sodium selenate plus ammonium sulphate—normal yield of grain. The plants in a fourth pot with no treatment were indistinguishable from those of *A*.

INHIBITION OF SELENIUM TOXICITY BY THE APPLICATION OF SULPHATES AND ELEMENTAL SULPHUR TO SOILS

Injury to plants grown in soils containing toxic concentrations of sodium selenate was inhibited both by the application of sulphates and of elemental sulphur. The effectiveness of ammonium sulphate when added with the selenate (20 p.p.m. selenium) to a clay-loam potting soil, when the plants were 3 months old and in the booting stage, is shown by figure 3. When selenium alone was added (fig. 3, *B*)

many of the leaves turned yellow in a week as though dying. No white chlorosis occurred because all the leaves had emerged at the time the selenium was added. Where ammonium sulphate was added with the selenium (fig. 3, *C*) the leaves remained normally green.

The plants were grown to maturity and the number of fertile heads per plant and the yield of grain recorded (table 4). Where selenium alone was added the heads were sterile. Where ammonium sulphate was added with the selenium the number of fertile heads and the yield of grain were almost normal.

TABLE 4.—*Average yield of grain of wheat plants in soil to which sodium selenate was added with and without ammonium sulphate*

[12 plants in each group]

Treatment	Fertile heads per plant	Weight of grain per plant
	Number	*Grams*
Selenium, 20 p.p.m	0	0
Selenium, 20 p.p.m.+$(NH_4)_2SO_4$	1.6	1.1
Control—no treatment	1.7	1.9
Control—$(NH_4)_2SO_4$ only	2.0	2.6

Other experiments showed the relation between the amount of sodium selenate added to the soil and the amount of ammonium sulphate necessary to inhibit the resulting chlorosis and stunting. In table 5 are recorded the relative degrees of injury obtained with two crops grown in the same soil, the first being sown in April and the second in the following October without additional treatment. Data from another experiment with similar soil containing 20 percent of sand, in which the selenate was more toxic, are recorded in table 6.

TABLE 5.—*Control of selenium injury to wheat plants (5 weeks old) by addition of ammonium sulphate to clay-loam soil* [1]

Selenium (in p.p.m.) as selenate	Injury to wheat plants grown in 6,000 g selenized soil treated with the following amounts (in grams) of ammonium sulphate—				
	0	1	2	3	4
	Seed sown Apr. 26, 1933				
15	++	++	+	0	0
20	+++	+++	++	+	0
30	++++	++++	+++	+++	+++
	Seed sown Oct. 2, 1933				
15	+	0	0	0	0
20	+++	++	+	+	0
30	++++	+++	+++	++	++

[1] Degree of injury indicated by number of plus signs.

TABLE 6.—*Control of selenium injury to wheat plants by additions of ammonium sulphate to clay-loam potting soil plus 20 percent of sand* [1]

[Oct. 6 to Nov. 6, 1933]

Selenium (in p.p.m.) as selenate	Injury to wheat plants grown in 6,000 g selenized soil treated with the following amounts (in grams) of ammonium sulphate—			
	0	2	4	6
10	+++	++	+	0
20	++++	+++	+++	++
30	Dead.	++++	+++	+++

[1] Degree of injury indicated by number of plus signs.

The data show the usual inverse relation between the amount of sulphate present and the toxicity of the selenate to the plants. Thus, in the April seeding (table 5) the chlorosis caused by 15 and 20 p.p.m. selenium in otherwise untreated soil was completely inhibited by applications of 3 and 4 g, respectively, of sulphate per pot. In the October seeding, where the injury from a given amount of selenium was somewhat reduced by the growth of the first crop, the injury from 15 p.p.m. selenium was inhibited by the presence of only 1 g of sulphate, 20 p.p.m. again requiring 4 g of sulphate for complete control. In the experiment recorded in table 6, where, owing to the greater toxicity of selenium in the sandy soil, only 10 p.p.m. selenium caused severe injury to the plants in the absence of excess sulphate, there was no chlorosis in the pots to which 6 g of sulphate had been added. These experiments show that in soils, as in nutrient solutions, injury from sodium selenate is determined by the relative amount of selenium with reference to the available sulphur.

Other experiments showed that not only ammonium sulphate, but all the sulphates that were tried, similarly inhibited the toxicity of sodium selenate. With equivalent applications of the phosphates and nitrates, as well as the sulphates of calcium, potassium, ammonium, and magnesium, only the plants in the pots treated with the four sulphates remained free from chlorosis and stunting, the rest being severely injured like those to which selenate only was added.

Elemental sulphur (after being in the soil a few weeks, or long enough to be oxidized to an available form) is similarly effective (fig. 4). In many experiments complete inhibition of the injury caused by selenium concentrations up to 20 p.p.m. has been obtained with 1 part of sulphur per 1,200 parts of the clay-loam soil (*A* and *D*) and definite improvement in the condition of the plants with rates of application as low as 1 part in 6,000 of soil. In soils in which the selenium was more toxic, larger applications of sulphur were required. The addition of calcium carbonate (12 g) to counteract the acidity resulting from the high sulphur applications did not interfere with control of symptoms of injury (*E*). Applications of gypsum also inhibited both the chlorosis and the stunting of the selenized plants (*F*).

DISCUSSION

Analyses of some of the material grown in these experiments by M. J. Horn, of the Bureau of Chemistry and Soils (using his modification of the Schmidt test for determining selenium in organic compounds (*5*)), have shown that chlorotic and obviously injured plants of both the sand and the soil cultures contained relatively large amounts of selenium in their tissues, whereas plants grown with

FIGURE 4.—Control of selenium injury to wheat produced by 20 p.p.m. selenium as sodium selenate in the clay-loam soil of the Arlington Experiment Farm, by the application of elemental sulphur and calcium sulphate: *B* and *C*, Sodium selenate only; *A* and *D*, sodium selenate plus 5 g sulphur (in 6,000 g soil); *E*, sodium selenate plus 5 g sulphur plus 12 g of calcium carbonate; *F*, sodium selenate plus 12 g of calcium sulphate.

these same concentrations of selenium, but with sufficient sulphates or sulphur to reduce or prevent the development of symptoms, contained markedly less. It seems, therefore, that absorption of selenium by the plants was determined not by its absolute concentration in the soil or nutrient solution but by its relative concentration with reference to the available sulphur.

Both the grain and the straw of wheat plants grown with but 1 p.p.m. selenium added to the soil as sodium selenate (an amount too small to cause visible injury to the plants) were extremely toxic to rats and guinea pigs in feeding tests conducted under the direction of E. M. Nelson, of the Bureau of Chemistry and Soils. The possibility that the toxicity to animals of plants grown in soils either naturally or accidentally contaminated with selenium can be prevented or reduced by the addition of excess sulphur or sulphates to the soil is now being investigated.

This selenium-sulphur relationship seems to constitute a hitherto unreported instance of antagonism. In nutrient solutions the toxicity of the selenate varied inversely with the amount of sulphate present, their numerical relationship at the point of minimum detectable injury being surprisingly reproducible. This phenomenon might be explained simply by the law of mass action, assuming (1) that selenium injury to the plant is due, as has been suggested (*3*), to replacement of the sulphur in some compound by selenium; and (2) that, in accordance with the proximity of selenium and sulphur in the periodic table, the mechanism responsible for the absorption of sulphur is unable to selectively exclude selenium, with the result that it is absorbed with sulphur in the same proportion that the two are available in the substratum. The further assumption that the proportion of affected molecules determines the visible effect on the plant would then furnish a possible explanation of the fact that wherever the available selenium in the nutrient solution is one-eighth or more of the available sulphur, many leaves of the plant are chlorotic, and that where it is only one-twelfth or less, the plant is not visibly affected, regardless of the absolute amount of selenium present (within limits). This explanation seems in harmony with Loew's observations (*13, p. 42*) concerning calcium-magnesium antagonism.

SUMMARY

The characteristic symptom of selenium injury produced in wheat plants by the addition of sublethal concentrations of sodium selenate (15 to about 30 p.p.m. selenium in Keyport clay loam) is a snow-white chlorosis, often accompanied, in sand cultures, by a pink coloration which varies in intensity from a pale tint to a rather deep rose-pink.

When the selenate is added to pots containing older plants the white chlorosis appears only on leaves emerging subsequent to the addition of selenium, those already formed merely turning yellow if the selenium concentration is high enough to affect them at all.

There is a progressive diminution of chlorosis on successive leaves as the plants become older, resulting in marked recovery of the plants.

The toxicity of sodium selenate is determined by the amount of sulphur available to the plants. In water cultures, selenium concentrations as low as 0.1 p.p.m. produced distinct injury after a few weeks with nutrient solutions containing no sulphate, whereas a concentration of 18 p.p.m. was required for this degree of injury in solutions containing 192 p.p.m. sulphur. There was no visible injury to the plants where the proportion of selenium to sulphur was 1:12 or less, the point of minimum detectable injury lying between 1:9 and 1:11. Where the ratio was 1:8 or greater the plants were chlorotic and stunted, and when the ratio was as high as 1:2, growth was almost completely inhibited.

Selenium injury to plants grown in soil in the greenhouse can be inhibited by the addition of excess sulphur, either in the form of sulphates or as elemental sulphur. The amount of sulphur or sulphate required for a given addition of selenate varies with the latter's toxicity in the particular soil.

LITERATURE CITED

(1) AWSCHALOM, M.
1921. DATOS SOBRE LA INFLUENCIA DEL SELENIO EN LA VEGETACIÓN CUANDO SUSTITUYE AL ION SULFÚRICO EN EL LÍQUIDO NUTRITIVO DE KNOP. INFLUENCIA DEL SELENÍTO SODICO EN LA VIDA DE LOS MICROORGANISMOS. Rev. Facult. Agron. La Plata 14: [122]–162, illus.

(2) BRENNER, W.
1916. ZÜCHTUNGSVERSUCHE EINIGER IN SCHLAMM LEBENDEN BAKTERIEN AUF SELENHALTIGEM NÄHRBODEN. Jahrb. Wiss. Bot. 57: [95]–127.

(3) CAMERON, C. A.
1880. PRELIMINARY NOTE ON THE ABSORPTION OF SELENIUM BY PLANTS. Roy. Dublin Soc. Sci. Proc. (n.s.) 2: 231–233.

(4) GNADINGER, C. B.
1933. SELENIUM. INSECTICIDE MATERIAL FOR CONTROLLING RED SPIDER. Indus. and Engin. Chem. 25: 633–637.

(5) HORN, M. J.
1934. QUALITATIVE METHOD FOR SELENIUM IN ORGANIC COMPOUNDS. Indus. and Engin. Chem., Analyt. Ed. 6: 34–35.

(6) HURD-KARRER, A. M.
1933. INHIBITION OF SELENIUM INJURY TO WHEAT PLANTS BY SULPHUR. Science (n.s.) 78: 560.

(7) KNOP, W.
1885. UEBER DIE AUFNAHME VERSCHIEDENER SUBSTANZEN DURCH DIE PFLANZE, WELCHE NICHT ZU DEN NÄHRSTOFFEN GEHÖREN. Ber. Verhandl. K. Sächs. Gesell. der Wiss. Leipzig, Math.-Phys. Cl. 37: [39]–54.

(8) LEVINE, V. E.
1914. REDUCTION OF SELENIUM COMPOUNDS IN THE LIVING ORGANISM. Biochem. Bull. 3: 463–465.

(9) ———
1915. BIOCHEMICAL STUDIES OF SELENIUM. Ann. N.Y. Acad. Sci. 26: 385–394.

(10) ———
1925. THE REDUCING PROPERTIES OF MICROÖRGANISMS WITH SPECIAL REFERENCE TO SELENIUM COMPOUNDS. Jour. Bact. 10: 217–262, illus.

(11) ———
1925. THE EFFECT OF SELENIUM COMPOUNDS UPON GROWTH AND GERMINATION IN PLANTS. Amer. Jour. Bot. 12: 82–90.

(12) LIPP, C. C.
1922. ALKALI DISEASE. Vet. Alumni Quart. [Ohio State Univ.] 10: 54–55.

(13) LOEW, O.
1899. THE PHYSIOLOGICAL RÔLE OF MINERAL NUTRIENTS. U.S. Dept. Agr. Bull. 18, 60 pp.

(14) LOUGEE, F. M., and HOPKINS, B. S.
1925. SELENIUM COMPOUNDS AS SPRAY MATERIALS. Indus. and Engin. Chem. 17: 456–459.

(15) NELSON, E. M., HURD-KARRER, A. M., and ROBINSON, W. O.
1933. SELENIUM AS AN INSECTICIDE. Science (n.s.) 78: 124.

(16) PETERS, A. T.
1904. A FUNGUS DISEASE IN CORN. Nebr. Agr. Expt. Sta. Ann. Rept. 17: 13–22; illus.

(17) ROBINSON, W. O.
1933. DETERMINATION OF SELENIUM IN WHEAT AND SOILS. Jour. Assoc. Off. Agr. Chem. 16: 423–424.

(18) STOKLASA, J.
1922. ÜBER DIE EINWIRKUNG DES SELENS AUF DEN BAU- UND BETRIEBSSTOFFWECHSEL DER PFLANZE BEI ANWESENHEIT DER RADIOAKTIVITÄT DER LUFT UND DES BODENS. Biochem. Ztschr. 130: [604]–643, illus.

(19) ———
1922. INFLUENCE DU SÉLÉNIUM ET DU RADIUM SUR LA GERMINATION DES GRAINS. Compt. Rend. Acad. Sci. [Paris] 174: 1075–1077.

(20) ———
1922. INFLUENCE DU SÉLÉNIUM SUR L'ÉVOLUTION VÉGÉTALE EN PRÉSENCE OU EN L'ABSENCE DE RADIOACTIVITÉ. Compt. Rend. Acad. Sci. [Paris] 174: 1256–1258.

(21) STOVER, N. M., and HOPKINS, B. S.
1927. FUNGICIDAL AND BACTERICIDAL ACTION OF SELENIUM AND TELLURIUM COMPOUNDS. Indus. and Engin. Chem. 19: 510–513, illus.

(22) TABOURY, [F.]
1932. SUR LA PRÉSENCE ACCIDENTELLE DU SÉLÉNIUM DANS CERTAINS VÉGÉTAUX. Compt. Rend. Acad. Sci. [Paris] 195: 171.

(23) TURINA, B.
1922. VERGLEICHENDE VERSUCHE ÜBER DIE EINWIRKUNG DER SELEN-, SCHWEFEL- UND TELLURSALZE AUF DIE PFLANZEN ... Biochem. Ztschr. 129: [507]–533, illus.

STUDIES OF DERRIS, NICOTINE, PARIS GREEN, AND OTHER POISONS IN COMBINATION WITH MOLASSES IN THE CONTROL OF THE GLADIOLUS THRIPS [1]

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INTRODUCTION

A spray containing paris green and brown sugar was the most effective of 14 insecticidal mixtures tested in the field against the gladiolus thrips (*Taeniothrips gladioli* M. and S.) in 1932 (*10*),[3] owing, apparently, to the persistence of a toxic residue on the foliage. In order to study more closely the action of this and other spray residues on various thrips, the investigations reported in this paper were started, and were carried out under laboratory and greenhouse conditions in 1932–33. Unless otherwise stated, the experiments were made on the gladiolus thrips.

METHODS

Three kinds of tests were made to estimate the effectiveness of various spray residues.

(1) Laboratory tests against young larvae hatching from sprayed foliage over a period of 6 to 8 days after the application: Potted gladiolus plants from 10 to 14 inches high were infested with from 10 to 15 adult female thrips 8 to 10 days before the application of the spray, in order to have many eggs in the foliage at the time of treatment. All thrips were removed from the plants before the spray was applied. After the spray deposit had dried, a band of sticky material was placed just above the leaf sheaths to prevent migration of the young larvae that had hatched from sprayed foliage. Figure 1 illustrates this method. Examinations and counts were made under a binocular microscope at 2-day intervals, and the dead larvae were removed. An effective spray residue kills large numbers of thrips larvae during or just after their hatching from sprayed foliage.

(2) Laboratory tests against older larvae and adult thrips: From 10 to 15 thrips were transferred from stock plants to 3- by ⅞-inch cotton-stoppered vials containing 3- to 4-inch strips of sprayed foliage. The vials were placed in a chamber with a constant temperature of 75° ± 1° F. Examinations and counts were made under a binocular microscope at 24-hour intervals. Small drops of water were added to the vials at regular intervals to keep the foliage fresh. In check tests young larvae were reared to maturity and adults usually survived from 120 to 144 hours, and longer if fresh foliage was added. Effec-

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[3] Reference is made by number (italic) to Literature Cited, p. 372.

tive spray deposits killed all larvae and adults in 48 to 72 hours. Tests were usually terminated after 72 hours. Figure 2 illustrates the vial-test method.

FIGURE 1.—Sprayed gladiolus plant used in testing the residual action of various sprays against thrips larvae hatching from eggs deposited in the leaf. The band of sticky material prevents migration of the young larvae up or down the leaf.

(3) Practical greenhouse tests: Heavily infested plants, 15 to 20 inches high, on which counts of the adult thrips on exposed foliage had been made, were sprayed (20 to 25 plants at a time) with a knapsack sprayer and then placed in large muslin cages in the green-

house. A second spray was usually applied 1 week later. The plants were also sprayed with water at low pressure to simulate the effect of rain. Counts of the number of thrips present on exposed foliage were made at intervals, and the effectiveness of the treatment was measured by the percentage of reduction in population.

The solutions or suspensions applied as sprays in this work usually consisted of the insecticide, supplementary material, and tap water. The concentration of the various materials is stated in the majority of cases by the ratio of 1 part of the substance to so many parts of water. Where the material is a liquid, such as nicotine sulphate or derris extract, the ratio is in parts by volume. Where the substance is solid, such as paris green or hellebore powder, the ratio is in parts by weight—a 1:400 ratio equaling 0.25 percent by weight or nearly 1 pound of material to 50 gallons of water. Where percentage concentrations are given the same rule holds true.

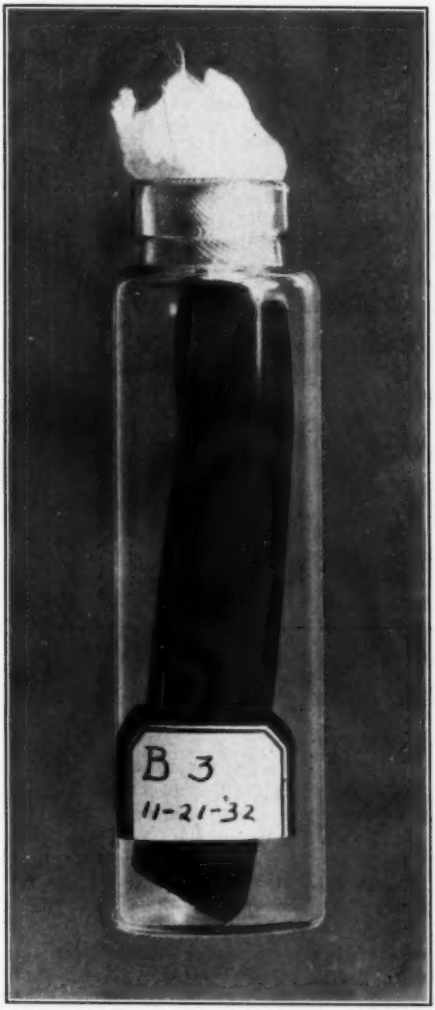

FIGURE 2.—Cotton-stoppered vial containing a piece of sprayed gladiolus leaf on which thrips adults or larvae have been placed for studying the action of various spray deposits on these insects.

NICOTINE AND NICOTINE SULPHATE [4] SPRAYS

Sprays containing nicotine sulphate and soap are widely used for thrips control. However, a spray of that type was among the least effective of those tried in 1932 against the gladiolus thrips (*10*). Some preliminary tests on the residues of this spray showed that it had little lasting action. The combination of waste sulphite liquor with nicotine sulphate killed a considerable number of thrips larvae hatching during a period of 3 to 4 days after the spray was applied. Tests with a number of different wetting agents were made, the results of which are given in table 1.

[4] Where nicotine sulphate is referred to in this paper it denotes the commercial product containing 40 ercent nicotine in the form of the sulphate.

TABLE 1.—*Toxicity of residues of various nicotine sprays to larvae of Taeniothrips gladioli hatching during a 7-day period after the application*

Spray [1]	Concentration of added substance	Total larvae	Mortality	Appearance of spray deposit
	Percent	*Number*	*Percent*	
Free nicotine alone		265	1.1	Spotty.
Nicotine sulphate with—				
Soap	[2] 0.25	268	.7	Thin, even film.
Sulphonated petroleum oil	.5	359	4.8	Do.
No wetting agent		326	12.2	Spotty.
Waste sulphite liquor	.75	493	30.0	Dropletlike.
Brown sugar	8.8	621	30.9	Do.
Petroleum white oil emulsion	[3] 1.0	626	31.4	Do.
Molasses	3.0	713	81.8	Do.
Untreated check		633	.2	

[1] Nicotine concentration 0.066 percent in all sprays, equivalent to 1:600 by volume of nicotine sulphate solution.
[2] Dry soap by weight.
[3] Oil by volume.

The effectiveness of the sprays seemed to depend both on the quantity of nicotine deposited on the foliage (as affected by the wetting agent used) and on its retention in the residue. Free nicotine was ineffective, probably because it disappeared quickly from the foliage by volatilization. Nicotine sulphate was more effective alone than with soap or sulphonated petroleum oil, possibly because these wetting agents left thin films on the foliage which probably contained less nicotine per unit area than the spotty coverage of the solution without spreader. The last four sprays listed in table 1 formed droplets on the foliage and were more effective. The petroleum oil emulsion droplets soon broke, forming a thin film of oil. Most of the larvae that were killed were those that hatched during the first few days after treatment. However, the nicotine sulphate-molasses spray, by far the most effective of the nicotine sprays tested, killed larvae over a longer period, indicating that nicotine was retained longer in the droplets of this combination than in the others. Figure 3 shows a larva killed by this spray deposit during the process of hatching.

Further tests by the first method were made of the nicotine sulphate-molasses combination with the same nicotine concentration and the same cheap baker's molasses at four concentrations, viz, 1, 3, 5, and 10 percent by volume. There appeared to be no advantage in using a concentration of molasses greater than 5 percent. Molasses solutions alone had no effect on the larvae. The last finding agrees with that of Hooker (*6*), who tested the sticking action of molasses on thrips.

Mixtures of nicotine sulphate with various types of molasses and sirup [5] were then tested by the first method, and a summary of the results is given in table 2. The hydrogen-ion concentration of all the molasses and sirup solutions was determined before and after the addition of nicotine sulphate.

[5] All sirups were diluted to register 5.5° Brix. This amounted to about 5 percent by volume.

TABLE 2.—*Effectiveness of nicotine sulphate with various types of molasses and sirup against the larvae of Taeniothrips gladioli hatching during a 7-day period after the application*

Type of sirup used with nicotine sulphate (1:600)	Hydrogen-ion concentration [1]		Total larvae	Mortality
	Before addition of nicotine sulphate	After addition of nicotine sulphate		
	pH	*pH*	*Number*	*Percent*
Cheap baker's molasses	5.9	5.83	305	78
Cuban blackstrap molasses	5.9	5.79	373	79
Louisiana blackstrap molasses	5.9	5.72	255	75
Sorgo sirup	5.5	5.45	328	76
Corn sirup	7.2	6.13	351	78
Honey	4.98	4.7	459	68
Tap water alone	7.25	6.1		

[1] Measurements made with a quinhydrone electrode that was checked on standard solutions before and after use.

All sirups except honey were about equally effective in combination with nicotine sulphate. Another series of tests confirmed the lower toxicity of the honey combination, which was more acid than the others and apparently wet the foliage less efficiently. All sirup solutions except that of corn sirup were slightly acid, and the addition of nicotine sulphate increased the acidity slightly.

FIGURE 3.—Larva of gladiolus thrips killed by the nicotine sulphate-molasses spray deposit during the process of hatching from the gladiolus leaf. The killing action took place before the antennae and legs had freed themselves from the enclosing embryonic cuticle (hatching membrane). Droplets of the spray deposit (dark areas) may be seen nearby and these are typical of the other molasses spray deposits on gladiolus. × 35.

Further tests by the first method with various concentrations of nicotine sulphate (1:400, 1:600, 1:1,000) in combination with a 5-percent solution of blackstrap molasses showed that mortality increased with increasing concentration of nicotine sulphate. The 1:400 concentration (0.1 percent nicotine) killed from 94 to 98 percent of the larvae hatching from sprayed gladiolus foliage during the period of 6 to 8 days after the application.

Although the nicotine sulphate-molasses residue killed large numbers of larvae at hatching, some larvae survived its action and grew to maturity, even though they were kept in close proximity to the spray deposit. Apparently the surviving larvae became resistant to nicotine. Campbell and his coworkers (*4*) have reported a similar reaction by mosquito larvae. Tests of the effectiveness of the spray deposit to larvae and adults in vial tests by the second method indicated that the molasses-nicotine sulphate residue was quite toxic to them shortly after the application. When tests were started 48 hours after the foliage was sprayed, however, only 17 percent of the

older larvae and 25 percent of the adults were killed, and after 8 days the residue was practically nontoxic.

The results of practical tests with this spray are shown in table 8. It gave considerable control as compared with the check, but it was far less effective than the paris-green or derris sprays. The relative ineffectiveness of this spray against older larvae and adults apparently accounts for its lower efficiency.

PARIS-GREEN SPRAYS

Blake and Connors (*1*) used effectively a combination of paris green with white sugar for the control of a thrips on roses. They found that a brown sugar-paris green spray produced slight foliage injury, but when molasses was substituted for the sugar severe foliage injury resulted.

However, since molasses was so much more effective than brown sugar in the nicotine-sulphate sprays (table 1), it was tried at 3-percent concentration in combination with paris green. Both thrips larvae and adults exposed to the molasses-paris green (1:400) residue on gladiolus were killed much faster than those exposed to the brown sugar-paris green deposit. Molasses, in addition to going into solution more easily than brown sugar, appears to wet the foliage better and covers it more thoroughly with droplets. Figure 4 illustrates this difference in molasses and brown-sugar spray deposits. Lower concentrations of paris green (1:400, 1:800, 1:1,600) were next tried in vial tests with both brown sugar and molasses. Here again the sprays with molasses were much quicker in action, killing all adults in 72 hours, whereas with brown sugar the kill produced by 1:800 and 1:1,600 paris green sprays was from 28 to 35 percent at the same time. Further vial experiments confirmed the foregoing tests, indicating that the paris-green concentration can be lowered to 1:1,600 (0.06 percent) when combined with molasses without losing its rapid and effective action, but with brown sugar such a reduction in paris green resulted in a considerable loss of potency. Three weeks after the application the residues were still about as toxic as when applied. Blackstrap molasses and baker's molasses were both used effectively. Cheap blackstrap molasses leaves a dark residue which might be objectionable on some ornamental plants. Here it might be preferable to use the higher priced lighter colored molasses, the deposits of which are not so noticeable. When the appearance of the deposit is of no concern, blackstrap can apparently be used just as satisfactorily.

Tests were next made to determine the role played by the various constituents of the sprays. The results of these tests indicated that neither molasses nor brown sugar alone was toxic to thrips and that paris green (1:400) alone was only slightly so. The filtrate from paris green alone was not very toxic when combined with molasses, but the filtrate from a mixture of paris green and molasses was nearly as toxic as the regularly prepared spray. Apparently the molasses has a definite connection with the production of the solution which is poisonous to thrips. At the suggestion of C. M. Smith, of the Bureau of Chemistry and Soils, it was decided to seek a chemical explanation of these results, and the following experiments were made with his cooperation. Mixtures of paris green (1:400) with tap water, brown-sugar solution, and molasses solution were prepared and agitated on a shaking machine for periods of ½, 2, and 8 hours. Immediately after

the bottles were removed from the machine the mixtures were filtered and then centrifuged to bring down the very fine material which passed through the filter. The plain water and the brown-sugar solutions showed practically no deposit in the centrifuge, but the molasses solution deposited an appreciable quantity of paris green. Evidently the molasses is a good deflocculator for paris green and so separates the finest particles that they go through the filter. Analyses

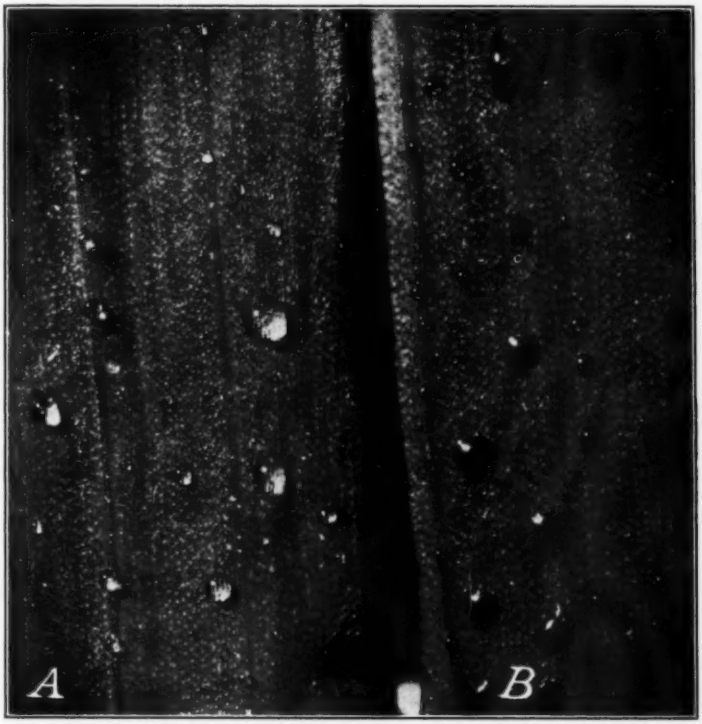

FIGURE 4.—Spray deposits on gladiolus leaf: Brown-sugar spray with rounded droplets (*A*); molasses spray with flattened droplets (*B*). × 12.

of soluble arsenic were made on the solutions after centrifuging; the results are shown in table 3.

It was quite evident that the solubility was greatly influenced by both time and the nature of the solvent. The quantity of soluble arsenic was much greater in the brown-sugar solution than in tap water alone and was by far the greatest in the molasses solution—this despite the fact that the molasses concentration was much lower than that of the brown sugar. Analysis for soluble copper in the molasses solution showed an amount approximately equivalent to the soluble arsenic present, indicating that the paris green had been dissolved as such. It is probable that the acidic constituents of molasses are largely responsible for the increased solubility of paris green. This increased solubility probably accounts for the greater toxicity observed in the molasses-paris green mixture.

TABLE 3.—*Solubility of paris green in tap water, brown-sugar solution, and molasses solution, as indicated by the quantities of soluble arsenic present after mixtures containing 2½ grams of paris green per liter had been agitated for different periods at about 22° C. (71.6° F.), then filtered and centrifuged*

Solvent	Period of extraction [1]	Soluble arsenic (As_2O_3)	
		Quantity per liter	Proportion of total paris green
	Hours	*Mg*	*Percent*
Tap water	½	7.9	0.32
	2	9.6	.38
	8	15.0	.60
Brown-sugar solution (88 g per liter)	½	45.0	1.80
	2	60.8	2.43
	8	97.3	3.89
Molasses solution (30 cc per liter)	½	94.4	3.78
	2	216.0	8.64
	8	378.0	15.12

[1] Period during which the mixture was agitated on a shaking machine. The 8-hour period represents a 2-hour period and a 6-hour period on successive days. Analyses were made by H. W. Rusk.

The question arose as to whether the molasses in the spray acts as a bait, but an experiment in which counts were made over several days of the number of thrips on sprayed and unsprayed plants (initial infestations equal) indicated that molasses had no baiting effect.

The results of the practical tests by the third method are given in table 8 and show that the molasses-paris green (1:800) spray and the brown sugar-paris green (1:400) spray were both very effective. A short time after the applications all plants sprayed with paris green showed some foliage injury, which was similar to, but more pronounced than, that noted in the field tests of 1932 (*10*). The high humidity prevailing in the cages in the greenhouse and the presence of considerable thrips-feeding injury on the plants when the tests were started may have helped to increase the arsenical burning. Brannon (*3*) noted that thrips injury intensifies arsenical burning on beans, and the same may be true of gladiolus.

Further tests by the third method with lower concentrations of paris green (1:800, 1:1,200, and 1:1,600) are given in table 4, and indicate that a concentration of paris green as low as 1:1,600, with molasses (1:33), would give good control under greenhouse conditions.

TABLE 4.—*Results of greenhouse tests of lower concentrations of paris green and molasses on Taeniothrips gladioli*

Concentration of paris green in 3-percent molasses solution	Initial infestation	Reduction in thrips population	
		7 days after first application [1]	12 days after second application [1]
	Number	*Percent*	*Percent*
1:800	40	92.5	100.0
1:1,200	83	95.5	98.8
1:1,600	111	94.5	97.3

[1] 7 days after the first application the plants were sprayed with water to simulate rain. After the plants had dried, a second application of the paris green-molasses sprays was made.

DERRIS SPRAYS

An acetone extract of derris containing 5.7 g of rotenone and 18.1 g of total extractives per 100 cc was used, the dilutions being prepared with acetone according to a method previously described (*9*). Tests of the first series were made with a 1:250 dilution of the derris extract to give 0.228 g of rotenone and 0.724 g of total derris solids per liter of 5-percent molasses solution. These tests indicated definitely that the deposit left by this spray was very toxic to all active stages of the thrips. Apparently the residue is quite stable, for it killed all larvae hatching from sprayed foliage over a period of 7 days after the application. A second and third series confirmed these results and indicated further that under greenhouse conditions considerable toxicity was retained for 2 and 3 weeks, though the residues were exposed during this time to sunlight coming through greenhouse glass. Lower concentrations of derris extract (1:250, 1:500, and 1:1,000, being approximately 1:5,000, 1:10,000, and 1:20,000, respectively, of rotenone) in combination with molasses killed all larvae hatching over the 7-day period after the application. The 1:1,000 concentration appeared to be somewhat slower in killing action than the higher concentrations. Results of tests on the lasting qualities of the residues are given in table 5.

TABLE 5.—*Stability of residues of derris extract-molasses spray under greenhouse conditions as indicated by tests with Taeniothrips gladioli*

Concentration of derris extract in 5-percent molasses solution	Effectiveness of residue [1]—	
	2 weeks after spraying	3 weeks after spraying
	Percent	*Percent*
1:250	93	50
1:500	74	43
1:1,000	56	21

[1] In terms of percentage of thrips killed in 72-hour vial tests in triplicate.

All the residues lost toxicity as time went on, but it was surprising that they retained toxicity so long, especially as it is known that rotenone and related products in derris extract are sensitive to light (*8*). Apparently the molasses in this spray helped to stabilize the derris residues, though it was also thought possible that the greenhouse glass did not transmit many of the rays which produce a deterioration of derris-spray deposits.

For this reason the effectiveness of derris extract alone and in combination with (1) soap, a distinctly alkaline wetting agent,[6] (2) sulphonated castor oil, an almost neutral wetting agent, and (3) molasses, a slightly acid agent, was investigated, the immediate effectiveness of these residues being first tried. The data are given in table 6.

[6] The liquid concentrate of potassium coconut-oil soap (near 40 percent soap) had a pH of 9.02, and a 1:100 dilution had a pH of 8.99. This soap contained excess free acid equivalent to 0.53 percent oleic acid. The pH determinations were made by H. E. Goresline and those of free acid by F. E. Dearborn, both of the Bureau of Chemistry and Soils.

TABLE 6.—*Toxicity of various derris-extract sprays to Taeniothrips gladioli*

[Tests in duplicate]

Derris extract (1:250) combined with—	Insects	Immediate effectiveness of residue to adults and larvae [1]	Insects	Mortality of larvae hatching during 7 days after spraying
	Number	*Percent*	*Number*	*Percent*
No wetting agent	33	78.7	107	88.8
Molasses (1:19)	33	100.0	658	100.0
Sulphonated castor oil (1:100)	33	85.0	122	82.0
Soap (0.33 percent) [2]	32	75.0	201	22.4
Check (untreated)	31	0		

[1] Residues tested on day following application, in a 72-hour vial test, in duplicate.
[2] Dry soap by weight.

All the derris sprays left residues which were quite toxic shortly after the application. The derris-molasses residue was definitely more effective and rapid in action than any of the others, the mortality being complete for both larvae and adults in 48 hours. (The figures in the third column of table 6 are for the 72-hour period.) All the sprays were of about the same efficiency against the hatching larvae, except the combination with soap, which was by far the least effective. Jones and Davidson (*7*) noted that rotenone suspensions are sensitive to the action of alkalies. Turner (*11*) found that cubé extract containing rotenone loses toxicity when allowed to stand with soap. The foregoing experiments indicate that soap continues to exert a deteriorating action on derris extract after the spray has dried on the foliage.

The stability of these residues when exposed to direct sunlight and to sunlight coming through greenhouse glass was next studied, one series of tests being made on residues exposed to 30 hours of sunlight over a 6-day period and one series on residues exposed to 85 hours of sunlight over a 14-day period. The data are shown in table 7.

TABLE 7.—*Toxicity to Taeniothrips gladioli of various derris residues after exposure to direct sunlight and sunlight coming through greenhouse glass*

[Method 2 in duplicate]

Derris extract (1:250) combined with—	Mortality of thrips caused by derris residue after—			
	30-hour exposure [1] to—		85-hour exposure [2] to—	
	Direct sunlight	Greenhouse sunlight	Direct sunlight	Greenhouse sunlight
	Percent	*Percent*	*Percent*	*Percent*
No wetting agent	18.6	41.5	0.0	4.3
Molasses (1:19)	82.1	97.7	79.0	93.2
Sulphonated castor oil (1:100)	11.9	43.2	0	17.2
Soap (0.33 percent by weight)	9.5	18.4	0	5.0
Check (untreated)		0		2.7

[1] Over a 6-day period.
[2] Over a 14-day period.

The derris-molasses residue was by far the most toxic, whether exposed to glass-filtered sunlight or to direct sunlight. After the 30-hour exposure the residues of derris extract alone and of derris with sulphonated castor oil were about equally toxic but were much less effective than the derris-molasses residue. Derris extract-soap residues had little toxicity whether exposed to direct sunlight or to that coming through glass. Exposure to direct sunlight caused a loss of toxicity in all residues, but this loss was least with the derris-molasses. After the 85-hour exposure over a 14-day period all spray residues except derris-molasses had lost practically all toxicity even under the greenhouse conditions. What little remained was entirely lost under exposure to direct sunlight. The derris-molasses residue alone retained a large percentage of its effectiveness.

Apparently molasses is responsible for the lasting powers of the derris spray on gladiolus. Whether this is due to a physical action of the molasses droplets in absorbing those light rays which produce deterioration or whether it is due to the use of a slightly acid medium is a question. The dropletlike residue undoubtedly makes possible the deposit of a considerable amount of spray with a relatively small exposed surface.

Extensive tests to determine the most effective type of molasses to use with derris extract have not been made, but the combination of the extract with either baker's molasses or cheap blackstrap has given very toxic residues.

Table 8 gives the results of the greenhouse tests with a derris-molasses spray in comparison with other molasses sprays. The tests indicate that under these conditions the derris spray would be very effective against the gladiolus thrips. Plants sprayed with this material were not injured by the treatment.

TABLE 8.—*Greenhouse tests (method 3) of various sprays used against Taeniothrips gladioli*

Spray	Initial infestation	Reduction (−) or increase (+) in thrips population—		
		7 days after first application [1]	7 days after second application [1]	10 days after second application
		Percent	*Percent*	*Percent*
Derris extract (1:250) with molasses (5 percent)	230	−95.6	−99.1	−93.9
Paris green (1:800) with molasses (3 percent)	275	−97.8	−98.5	−95.6
Nicotine sulphate (1:600) with molasses (5 percent)	181	−62.4	−58.5	+21.5
Paris green (1:400) with brown sugar (8.8 percent)	274	−92.3	−99.3	−92.7
Check (untreated)	198	+4.0	+66.5	+46.5

[1] 7 days after the first application the thrips were counted and the plants then sprayed with water to simulate rain. After the plants had dried, all sprays were again applied to their respective plants. Another count was made and a water spray applied 7 days later, but the sprays were not renewed after this. A third count was made 3 days later.

In the field tests of 1933 the derris-molasses spray appeared to be somewhat less effective as compared with the paris-green sprays than might have been expected from the greenhouse tests (unpublished data of R. H. Nelson). Several fungi (mostly *Cladosporium*) [7] were found attacking the derris-molasses deposit, and it is possible that they may have affected the efficiency of this spray. Further study

[7] A small amount of *Alternaria* and some *Penicillium* were also present. The indentifications were made by L. McCulloch, of the Bureau of Plant Industry.

of this spray for use under field conditions is needed. The fungi apparently did not attack the paris-green sprays.

Against a thrips (*Thrips nigropilosus* Uzel) on cinerarias in a greenhouse, the derris-molasses spray exerted a residual action and gave commercial control. Against the onion thrips (*Thrips tabaci* Lind.) on onion it was not so effective, apparently because the spray did not penetrate and wet the young center leaves ("chits") where the majority of the insects congregate. With an efficient wetting agent, such as sulphonated castor oil (1:400), in place of the molasses, the derris extract appeared to be much more effective in reducing the thrips population than the nicotine sulphate-soap spray in greenhouse and small field tests. Under greenhouse conditions this spray deposit still exerted a toxic action, 8 days after application, to *T. tabaci* larvae and adults, similar to that exerted against the gladiolus thrips. The deposit left by the nicotine sulphate-soap spray had little or no toxicity to the onion thrips.[8] This derris spray has also shown considerable effectiveness against the red spider mite (*Tetranychus bimaculatus* Harvey) on rose, killing all stages, including the egg, when it is applied thoroughly.

HELLEBORE SPRAYS

Hellebore powder as a dust or diluted 1:25 in water has been reported by Hamilton (*5*) as being effective against the gladiolus thrips.

In the tests described herein, hellebore was obtained as the root of *Veratrum album*. It was ground into a fine powder and analyzed (1.3 percent alkaloids). This powder was tested at 1:67 (equaling about 6 pounds to 50 gallons) of 5-percent blackstrap molasses solution. Under greenhouse conditions the deposit left by the spray killed all thrips hatching over a 7-day period after the application, and it appeared quite stable, as all adults and larvae were killed in vial tests made on the residue 7 days after spraying. Lower concentrations of hellebore (1:100, 1:200, and 1:400) in molasses solution were all found to be very toxic as much as 9 days after the application. In a small greenhouse test (method 3) one application of hellebore at 1:400 with 5-percent molasses solution reduced the population 94 percent in 12 days, but the 1:800 concentration was apparently too low to be effective (47.2 percent reduction). In later tests the hellebore (1:400) molasses spray to which sulphonated castor oil (1:400) had been added for wetting purposes appeared to be quite effective against *Thrips tabaci* in greenhouse and field tests on onion.

PYRETHRUM SPRAYS

Alcoholic extract of pyrethrum, diluted to contain 1 part of pyrethrin I in 10,000 parts of water,[9] was tested, in combination with 3-percent molasses solution. Three hours after the application the deposit left by this spray showed some toxicity to *Taeniothrips gladioli* larvae and adults, but its toxicity disappeared after 24 hours, under

[8] The writer has recently received a published summary in which Bourne (*2*) reports that a commercial derris spray was slower in its initial action against *Thrips tabaci* on onion than nicotine sulphate sprays, but exerted a residual effect, and that nicotine sulphate gave the greatest reduction in thrips population.

[9] Analyses of the concentrate showed 0.68 g pyrethrin I per 100 cc at the time the insecticide tests were made. A dilution of 1.42 cc of alcoholic concentrate to 98.6 cc of spray gave a 1:10,000 concentration of pyrethrin I. Recent analyses of this same concentrate by the copper reduction method showed 1.34 g of total pyrethrins per 100 cc; so the concentration of total pyrethrins in the sprays used in the tests was at least 1:5,000.

greenhouse conditions. Its effectiveness against hatching larvae was also very poor, only 4 to 7 percent being killed in two tests against 910 larvae. The combination with waste sulphite liquor (1:133) was equally inefficient (5 percent of 399 larvae). Pyrethrum powder (0.32 percent pyrethrin I) at the rate of 1:33 with 5-percent molasses solution, was also ineffective (0.2 percent of 417 larvae). Although pyrethrum extract sprays have considerable contact action against thrips, their spray deposits had little toxicity at the concentrations tested.

MISCELLANEOUS SPRAYS

Tartar emetic, at 1:400 with 3-percent molasses solution, was very toxic to adult thrips. Borax, at 1:200 with 5-percent molasses solution, left a deposit which, though slower in action than paris green, gave a very high mortality, but it produced foliage injury. Calcium arsenate, at 1:533 and 1:266 (about 1½ or 3 pounds, respectively, per 100 gallons) with 5-percent molasses solution, showed moderate toxicity to all active stages of thrips, but was much slower in action than the paris green-molasses residue. The deposits left by lead arsenate (1:200) and copper sulphate (1:100), each in combination with molasses, had some toxicity but were very slow in action.

DISCUSSION

The experiments described in this paper have brought out the importance of maintaining a toxic spray residue on foliage for the control of thrips. Such a spray residue is especially important in the control of a thrips like *Taeniothrips gladioli* on gladiolus, which is on the exposed foliage part of the time but spends considerable time deep in the leaf sheaths beyond the reach of a contact spray. Pyrethrum, because of the instability of its residue, appears to have value only as a contact poison and does not act against thrips over an extended period. Derris extract, hellebore, nicotine sulphate, and paris green and the other inorganic poisons tested, when combined with molasses in sprays, leave residues that remain effective for various periods of time, the inorganic poisons being the most stable. Probably the hooking, gashing, sucking method of thrips feeding accounts for the toxic action of the residues, though the deposits may also act as contact insecticides. These residues are apparently easily washed off by rain. Only the paris-green residues (of those exposed to rain) retained considerable toxicity after a precipitation of 0.6 inch.

SUMMARY

The deposit left by the nicotine sulphate-molasses spray killed many hatching thrips larvae over a 7-day period after the application and was the most effective of 8 nicotine spray residues tried. It was quite toxic to older larvae and adults shortly after application, but soon lost its effectiveness and was not very promising in greenhouse control tests.

Paris green (0.125 percent (1:800) or less) was more toxic with molasses, either blackstrap or higher grades, than with brown sugar. Chemical analyses of these mixtures corroborated these results, indicating that paris green is much more soluble in molasses solution than in brown-sugar solution. Its solubility in tap water alone was much

less than in either of these solutions. Both the brown sugar-paris green (1:400 or 0.25 percent) deposit and the molasses-paris green (1:800 or 0.125 percent) deposit retained toxicity for some time, even after exposure to rain, and both were very effective in greenhouse control tests, though some foliage injury resulted, especially on plants with considerable thrips feeding. Molasses did not appear to act as a bait.

Derris extract with molasses solution left a very toxic deposit and was very effective in greenhouse control tests. The residues of derris extract, alone or in combination with soap or sulphonated castor oil, were quite toxic shortly after the application, but lost toxicity much faster (especially the soap spray) than derris-molasses residue when exposed to direct or to glass-filtered sunlight. The derris-molasses deposit was easily washed off by rain and under field conditions it was attacked by a fungus. Under greenhouse conditions it gave commercial control of *Thrips nigropilosus* Uzel on *Cineraria*. It was not effective against *T. tabaci* Lind. on onion. In greenhouse and small field tests derris extract with sulphonated castor oil was more promising for onion thrips control than nicotine sulphate and soap. The same derris spray has also shown promise in red spider mite control on rose and other plants.

Hellebore powder with molasses solution left a toxic and fairly stable deposit and showed considerable effectiveness against the gladiolus thrips. On addition of sulphonated castor oil, it gave promising results against the onion thrips.

Pyrethrum extract spray with molasses or waste sulphite liquor left a deposit of slight immediate toxicity, which disappeared quickly. Results with tartar emetic, borax, calcium arsenate, lead arsenate, and copper sulphate are also reported.

These experiments indicate that the toxicity and stability of the spray deposit are important factors in thrips control.

LITERATURE CITED

(1) Blake, M. A., and Connors, C. H.
1915. spraying experiments to control thrips upon roses in the greenhouse. N.J. Agr. Expt. Sta. Ann. Rept. (1914) 35: 39–40.

(2) Bourne, A. I.
1933. control of onion thrips. Mass. Agr. Expt. Sta. Bull. 293: 30.

(3) Brannon, L. W.
1932. observations on the association of thrips with arsenical injury on snap beans. Jour. Econ. Ent. 25: 1112–1113.

(4) Campbell, F. L., Sullivan, W. N., and Smith, C. R.
1933. the relative toxicity of nicotine, anabasine, methyl anabasine, and lupinine for culicine mosquito larvae. Jour. Econ. Ent. 26: 500–509, illus.

(5) Hamilton, C. C.
1933. greenhouse and field tests for the control of the gladiolus thrips (taeniothrips gladioli m. & s.). Jour. Econ. Ent. 26: 555–564.

(6) Hooker, W. A.
1907. the tobacco thrips, a new and destructive enemy of shade-grown tobacco. U.S.Dept.Agr., Bur. Ent. Bull. 65, 24 pp., illus.

(7) Jones, H. A., and Davidson, W. M.
1931. preparations containing rotenone for use as insecticides. i. aqueous suspensions. Jour. Econ. Ent. 24: 244–257.

(8) Jones, H. A., Davidson, W. M., Gersdorff, W. A., Gooden, E. L., Campbell, F. L., and Sullivan, W. N.
1933. loss in toxicity of deposits of rotenone and related materials exposed to light. Jour. Econ. Ent. 26: 451–470, illus.
(9) Richardson, H. H.
1933. preparation of derris extract sprays. Jour. Econ. Ent. 26: 995.
(10) ——— and Nelson, R. H.
1933. field control of the gladiolus thrips (taeniothrips gladioli m. & s.). Jour. Econ. Ent. 26: 546–554.
(11) Turner, N.
1932. notes on rotenone as an insecticide. Jour. Econ. Ent. 25: 1228–1237, illus.

THE FORMS OF NITROGEN IN INFUSIONS OF CORN, TIMOTHY, RED CLOVER, TOBACCO, AND RED TOP[1]

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INTRODUCTION

In view of the probable variety of decomposition products of plants when subjected to decomposing agents the writer attempted to discover the fate of the nitrogen of plants during the period of infusion. The products of diffusion were probably modified to a degree by water and by the presence of toxic bodies arising from the action of bacteria during the early stages of infusion.

The investigation was also suggested by a preliminary examination of soils of tobacco fields affected with brown root rot and those not affected. The affected fields had had corn planted on them in the preceding years, while the nonaffected fields had had tobacco planted on them. It was found that soil from the fields affected with "brown rot" contained a greater amount of carbon. This would suggest that decomposition of plant substance went on more slowly in fields in which corn had been planted the preceding year. The affected soil also reduced potassium bromate more completely, which would indicate the presence of an increased amount of reducing substance, formed, perhaps, during decomposition. The differences in the carbon content, or the reducing action, of the soils from these fields were not great, but were consistent, as was indicated in a comparison of two other fields under the same conditions.

Adjacent fields were chosen in order that the soil type and other environmental factors might be as nearly uniform as possible.

It was not assumed that infusions were like soil extracts. The study was made to determine whether there is any analogy between nitrogen derivatives in infusions of specific plants and the results obtained in the field.

REVIEW OF LITERATURE

Our ideas of soil toxins arising from plant residues have been simplified by the isolation of specific compounds, principally labile, which are affected by heat, relative lack of water, access of air to soil, and other factors.

Collison[2] found that vanillin and dihydroxystearic acid were present in ether extract of wheat. The latter compound was found also in timothy. From alfalfa hay vanillin and salicylic acid were extracted.

[1] Received for publication Mar. 14, 1934; issued August 15, 1934. Contribution no. 190 of the Massachusetts Agricultural Experiment Station.

[2] COLLISON, R. C. THE PRESENCE OF CERTAIN ORGANIC COMPOUNDS IN PLANTS AND THEIR RELATION TO THE GROWTH OF OTHER PLANTS. Jour. Amer. Soc. Agron. 17: 58-68. 1925.

Doran [3] was able, by making water infusions of timothy plants and adding them to soil, to induce either harmless or decidedly harmful effects, depending on the age and consequent stage of decomposition. Infusions 5 weeks old or less caused little injury to tobacco plants, while those 7 weeks old exerted a decidedly deleterious effect on the plants, causing lesions and discoloration not unlike the brown roots of tobacco when grown following timothy, corn, or clover in the field.

Breazeale [4] from his investigations on residual portions of sorghum decomposition products in the soil concluded that the deleterious effects were due to toxic bodies. These bodies were readily subject to change in composition and physiological effect. He found that these bodies were sufficiently virulent to cause the death of wheat seedlings if introduced in the cultural solution within a few hours after they were recovered by distillation.

METHODS

The plants used for infusions were corn, *Zea mays* L. (in the form of dry and green stover); timothy, *Phleum pratense* L.; red clover, *Trifolium pratense* L.; tobacco, *Nicotianum tabacum* L.; and redtop, *Agrostis palustris* Huds. All were approximately in the stage of maturity as when usually harvested. The one exception was dry corn stover which had been kept in a barn during the winter. Redtop, like tobacco, has been found less harmful to a following tobacco crop than are the other plants employed. The corn stover and tobacco were chopped up. The materials were weighed green, and samples were taken for dry-weight determination. In each case about 6 pounds of plant materials (green weight) was placed in a crock and a measured amount of water added. The crocks were allowed to remain in the greenhouse during the entire infusion process. Calculations were made on the basis of original dry weight. At the end of 7 weeks aliquots of the infusion material were filtered through cloth and used for nitrogen determinations. The solutions, or infusions, were then analyzed for the following nitrogen components:

In determining the total nitrogen, 50 cc of the infusion was made acid with sulphuric acid and taken to dryness on a water bath. The modified method of Ranker [5] was then followed.

For ammonia nitrogen 100 cc of the infusion was made alkaline with calcium oxide and the ammonia distilled into standard acid (0.02N) by steam distillation.

Protein nitrogen was determined by precipitation with Stutzer's reagent. It might be stated that up to the present time no perfect reagent for protein determination in the presence of proteose has been suggested.

For proteose nitrogen the compounds were determined by making an aliquot portion from the protein filtrate slightly acid with sulphuric acid, and saturating the solution with zinc sulphate. The precipitate was filtered and treated by the usual Kjeldahl method.

The amides were determined as follows: To 200 cc of protein sample of filtrate was added sufficient sulphuric acid to make it

[3] DORAN, W. L. THE GROWTH OF TOBACCO AND BROWN ROOT ROT OF TOBACCO AS AFFECTED BY TIMOTHY INFUSIONS OF DIFFERENT AGES. Jour. Agr. Research 36: 281-287. 1928.

[4] BREAZEALE, J. F. THE INJURIOUS AFTER-EFFECTS OF SORGHUM. Jour. Amer. Soc. Agron. 16: 689-700, illus. 1924.

[5] RANKER, E. R. DETERMINATION OF TOTAL NITROGEN IN PLANTS AND PLANT SOLUTIONS: A COMPARISON OF METHODS WITH MODIFICATIONS. Ann. Mo. Bot. Gard. 12: 367-380, illus. 1925.

equal to a 5-percent solution. The filtrate was hydrolized for 2 hours by boiling, a reflux condenser being used. At the end of the hydrolysis period the solution of ammonia salts was made alkaline with calcium oxide, and the ammonia was driven over by steam distillation into standard acid (0.02N).

Humin nitrogen was determined by taking the residual material in the flask from which the hydrolyzed amide ammonia had been distilled, filtering it while hot and treating the residue by the Kjeldahl method used in the protein and proteose determinations.

After removing ammonia nitrogen from a portion of the infusion the alpha amino acid nitrogen was determined by using the Van Slyke apparatus.

No nitrate nitrogen could be detected in any of the infusion mixtures.

RESULTS AND DISCUSSION

Judging from the amount of undecomposed plant tissue left after filtering, the infusions of corn, timothy, and clover varied little in the rate of decomposition. Tobacco was apparently most nearly decomposed, and redtop seemed to be most resistant to infusion. These results were verified by the amounts of total nitrogen found in each sample. Tobacco infusions contained the most total nitrogen; corn, timothy, and clover, intermediate amounts; and redtop by far the least of all the plants used.

The most pronounced difference in the samples was in the variety of nitrogen compounds present (table 1). All infusions contained ammonia, protein, and proteose nitrogen. Corn, timothy, and clover infusions contained all the general nitrogen derivatives, and smaller portions of ammonia than tobacco. Clover was higher than timothy or corn in ammonia content. Dry corn stover contained the least ammonia, and redtop came next. Tobacco and redtop infusions contained no humin nitrogen, and only traces of alpha amino acid and amide nitrogen.

TABLE 1.—*Percentages of nitrogen in various forms found in water infusions of different plants*

[Results computed on dry-weight basis]

Plant	Form of nitrogen						
	Total	Ammonia	Protein	Proteose	Amide	Alpha amino acid	Humin
Corn stover (dry)	0.5402	0.0015	0.1786	0.0251	0.0988	0.1041	0.0871
Corn stover (green)	.3081	.0805	.1403	.0044	.0291	.1255	.0208
Timothy	.5580	.1577	.1299	.....	.1363	.1135	.0205
Red clover	.5529	.2654	.1514	.0321	.0402	.0417	.0321
Tobacco	1.1650	.7702	.3718	.0048	.0315	[1]	.0000
Redtop	.0407	.0240	.0116	.0043	[1]	[1]	.0000

[1] Trace.

The percentages of the total nitrogen of the infusion, in the form of ammonia nitrogen, are approximately as follows: Dry corn stover, 0.27 percent; green corn stover, 20 percent; timothy, 28 percent; red clover, 48 percent; tobacco, 66 percent; and redtop, 58 percent. These figures represent approximately the rapidity of ammonification under the conditions of the experiment. Tobacco was nearest to com-

plete ammonification; corn was in the most incomplete stage. It seems rational to suppose that in humid areas the rate of ammonification in most soils would represent approximately the rate of nitrate formation.

The following percentages of the total nitrogen are in the form of protein: 33 in dry corn stover, 35 in green corn stover, 23 in timothy, 28 in red clover, 31 in tobacco, and 28 percent in redtop. Corn infusions contained the highest percentage of protein of the infusions tested. The percentages of the total nitrogen of the infusions that remained in the form of protein nitrogen would represent approximately the inverse of the rate of decomposition under the conditions of the experiment.

Of all the infusions, corn and timothy had the highest percentages of their total nitrogen in the form of amides and alpha amino acids. Corn and timothy may be grouped together with respect to low percentages of total nitrogen in the form of ammonia and high percentages as alpha amino and amide nitrogen. Tobacco and redtop, on the other hand, have relatively high percentages of the total nitrogen of their infusions in the form of ammonia, and contain only traces of alpha amino acids and amides. Red clover stands intermediate between these two groups with respect to the distribution of its nitrogen compounds.

That ammonia is an end product in this type of experiment can fairly well be contended. Those plants in which the nitrogen arrives at this state most directly are less likely to have nitrogen residues which may, with carbohydrates present, give rise to harmful compounds.

No analyses were made of plants before infusion, but the total nitrogen of such plant materials is well known. The redtop and timothy contained slightly more than 1 percent of nitrogen by dry weight; clover, slightly more than 2 percent; corn stover, slightly less than 1 percent; and tobacco varied with respect to its total nitrogen content. It is thus apparent that approximately one-half of the total nitrogen of corn, timothy, and tobacco, and less of clover and redtop was retained in some form in the infusion at the end of the 7-week period. Probably most of the discrepancy is due to the fact that a large part of the nitrogen of the plant tissue did not go into the infusions. There is also a strong probability that under the anaerobic conditions existing some nitrogen had passed off into the air.

SUMMARY

Water infusions were made of corn stover (green and dried), timothy, red clover, tobacco, and redtop. The duration of the infusion period was 7 weeks.

All the plant infusions at the time of analysis contained ammonia, protein, and proteose nitrogen.

Corn and timothy showed low percentages of their total nitrogen in the form of alpha amino and amide nitrogen; tobacco and redtop had high percentages of total nitrogen in the form of ammonia and only traces of alpha amino acids and amides; red clover was intermediate between the two groups in the distribution of its nitrogen compounds.

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